ZINC AND MAGNESIUM LEVEL AND ITS ASSOCIATION WITH GLYCATED HEMOGLOBIN IN TYPE 2 DM" - A CROSS SECTIONAL STUDY

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CERTIFICATE

This to certify that the dissertation entitled **"ZINC AND MAGNESIUM LEVEL AND ITS ASSOCIATION WITH GLYCATED HEMOGLOBIN IN TYPE 2 DM" – A CROSS SECTIONAL STUDY** is the bonafide original work done by **DR.M.S.GAYATHRI** , Post graduate in **Biochemistry under** overall supervision and guidance in the Department of Biochemistry, Kilpauk Medical College, Chennai , in partial fulfillment of the regulations of The Tamilnadu Dr. M.G.R . Medical University for the award of M.D.Degree in Biochemistry **(Branch XIII)**

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DECLARATION

I solemnly declare that this dissertation entitled **"ZINC AND MAGNESIUM LEVEL AND ITS ASSOCIATION WITH GLYCATED HEMOGLOBIN IN TYPE 2 DM" – A CROSS SECTIONAL STUDY** was written by me in the Department of Biochemistry, Kilpauk Medical College, Chennai, under the guidance and supervision of **Prof. DR.V.MEERA, M.D.,** Professor & HOD, Department of Biochemistry & Kilpauk Medical College, Chennai – 600010.

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CONTENTS

ABBREVIATIONS

DM	-	Diabetes Mellitus
Th1 cell	-	T Helper 1 cell
NK cell	-	Natural killer cell
HIV	-	Human Immuno deficiency virus
AIDS	-	Acquired immuno deficiency syndrome
HbA1c	-	Glycated hemoglobin
ADA	-	American Diabetic Association
IDDM	-	Insulin Dependent Diabetes Mellitus
NIDDM	-	Non Insulin Dependent Diabetes Mellitus
GDM	-	Gestational Diabetes Mellitus
IGT	-	Impaired Glucose Tolerance
IFG	-	Impaired Fasting Glucose
OGTT	-	Oral Glucose tolerance Test
BMI	-	Body Mass Index
IRS	-	Insulin Receptor Substrate Protein
PI $_3$K	-	Phosphatidyl Inositol 3 –Kinase
GAD	-	Glutamic Acid Decarboxylase
ICA_S	-	Islet cell Cytoplasm Antibodies
IAA_S	-	Insulin Auto Antibodies
Zn T8	-	Zinc Transporter 8
HOMA-IR	-	Homeostatic Model Assessment for Insulin Resistance

INTRODUCTION

‘Diabetes mellitus is a chronic disorder of various metabolism involving carbohydrate, fat and protein. It is associated with many causes which end in chronic hyperglycemia. The cause may be due to insufficient insulin secretion, or insulin action, or both.[1] these may end in long term complications, and failure of multiple organ systems. Death is due to acute metabolic complications. The chronic disease will lead to irreversible physiological and anatomical changes in various tissues in the body, but mainly in the vascular system.

A correlation was noticed among diabetes and micro nutrients like magnesium, vanadium, manganese, zinc and selenium [2]. A mechanism which is accepted explains, that enhancement of insulin action at the receptor level occurs by these micro nutrients[3]. They act as cofactors or part of the enzyme system, needed for the citric acid cycle in the carbohydrate metabolism. These minerals behave as antioxidants and prevent lipid per oxidation. It also stimulates the biological action of insulin[4].The main complication of type 2 DM is an elevated blood glucose level. The action of zinc is based on its enzymatic affinity and its metalloenzymecomplex[4]which is needed for the secretion and sorting of insulin .zinc enhances the structural integrity of biological receptors of insulin. The central role of zinc is in cell division and protein synthesis. It is mainly needed for the growth of infants and adolescents.

In pregnant women it is needed for the growth of the fetus. The deficiency in this group is due to poor intake of food which is rich in zinc.

Magnesium has an essential role in carbohydrate metabolism. Hypomagnesaemia causes altered phosphorylations in citric acid cycle. So mineral deficiency may lead to a disease condition or it may be either way[5]

Magnesium is one of the important intracellular cation in the body. It acts as a cofactor for enzymatic reactions involved in carbohydrate metabolism, nucleic acid and protein synthesis[6] Magnesium is one of the essential macro mineral and is associated with glucose intolerance , insulin release and insulin resistance in experimental animals and humans. Hypomagnesaemia is a more common finding in diabetes patients.

Clinically, zinc deficiency shows growth retardation, delayed sexual and bone maturation ,skin patches , diarrhea, alopecia, decreased appetite, more vulnerability to infections due to defects in the immune system, and changes in behavior .Mild zinc deficiency is implicated in diseases, like, HIV/AIDS, diabetes , alcoholism, cirrhosis and inflammatory bowel disease, zinc is linked with several aspects of immune system. Development of B lymphocytes, T-Helper 1(Th1) cytokine production and NK cell Th1 production of antibodies, and cytolytic activity are affected mostly [6] Neutrophil, macrophage functions are adversely affected by zinc deficiency. Apoptosis of lymphocytes are initiated due to zinc deficiency. Zinc also acts as an antioxidant and thus can play a prime role in stabilization of cell membranes[6].

Constantly elevated blood sugar level will lead to glycosylation of the proteins primarily hemoglobin .Hemoglobin glycation, measured by percentage of glycated hemoglobin (HbA1c) was done 30 years ago to estimate the degree of chronic hyperglycemia in diabetic patients. The results reflect the average glucose levels over the preceding three months. In Diabetics an elevation of HbA1c of 1 percent increase will lead to 30% in mortality associated with cardiovascular events[7]. So in this study, evaluation of zinc and magnesium level and its association with glycated hemoglobin was decided to be studied.

AIM AND OBJECTIVES

To assess the level of zinc and Magnesium and its levels are compared with Glycated Hemoglobin in type2 DM patient and in normal subjects. The secondary objective of the study is Improvement in Zinc and Magnesium level in diabetic patients will have a better glycemic control which in turn will prevent the complication and progression of the disease.

REVIEW OF LITERATURE

Diabetes mellitus (DM) is a metabolic condition, in which chronic hyperglycemia is associated with relative or complete absence of insulin secretion. It may be due to a defect in the mechanism of action of insulin or a defect in the receptor activity[1]. The persistently elevated blood sugar will lead to multisystem involvement which will eventually end in multi -organ failure. The Multi- organ dysfunction will be the cause for the raised morbidity and mortality in type 2 DM [2]

The symptoms are Polyphagia, polydypsia, polyuria which ends in weight loss. Because of severe hyperglycemia the lens is most affected, leading to blurred vision .The defect in development is manifested as impairment of growth and vulnerability to infections. Acute metabolic acidosis is the most serious complication of persistent hyperglycemia. Chronic complications are mainly due to involvement of micro vascular system[3]

The complications are chiefly due to deposition of lipid in the micro vessels of important organs like retina, heart, kidney and nerves. It not only involves blood vessels and also involves every system and each and every tissues of the body. In the lower limbs especially the foot ulcer in various forms may lead to amputations at different levels. Charcot joints, autonomic neuropathy causing gastrointestinal, genitourinary, sexual, cardiovascular and peripheral vascular symptoms and disorders.

DIABETES MELLITUS EPIDEMIOLOGY

About 3.2 million worldwide deaths occur due to diabetes. Diabetes is a common health disorder throughout the world and its frequency rising dramatically. According to the WHO, At least 171 million people in the world have been diagnosed as diabetics. This number is likely to double by 2030 [7].The situation is worse in developing countries. Every year the death rate is increasing and one in every 20 deaths is attributable to diabetes and its complication. In the year 2000 approximately 6% of deaths were due to diabetes-associated complications. ADA proposes that, about "18.3% (8.6 million)" of people at the age of 60 yrs and older had type 2 DM. NHANES -III (The National Health and Nutrition Examination Survey) reveals, that population over 65 years old have diabetes with 18% to 20% and 40% are in the pre diabetic stage of . A population based study by AHRQ (agency for health care research and quality) says that complications of type 2DM is one of the 20 main expensive situations seen in U.S inpatient hospitalization.

India ranks first with highest number in the prevalence of Type 2 Diabetes mellitus and China coming second with 20.9 million people. In India, nation-wide surveillance study has found that the prevalence of type 2 diabetes mellitus in urban population is about 7.3 % and in peri-urban/rural areas it is 3.2%. In Chennai the prevalence of diabetes mellitus is 13.5% in 2000, which increased steeply to 14.3%, in 2004 and further increased to 18.6% in 2006. In India the death due to diabetes is about 1 million. In India the average age of

onset of diabetes is about 42.5 years. The higher incidence of diabetes in India is due to people going in for high calorie diet, genetic susceptibility and sedentary life style[7]

MICRONUTRIENTS AND DIABETES MELLITUS

Micronutrients are important nutrients that are required in tiny amounts for the day to day function to occur properly.[8] It includes four major groups.

1. Vitamins
2. Major elements or macro minerals
3. Trace elements or minor elements or micro minerals
4. Organic acids

When the daily requirement is more than 100mg/day they are called as macro minerals or major elements. If the requirement is less than 100mg/day, it is called minor elements or micro minerals. Deficiency of micronutrients affects more than 50% of population which results in increased mortality and morbidity rates. It also results in loss of productivity and permanent impairments of cognitive function in infants and children.

Macro minerals include iron, chloride, sodium, calcium, magnesium, phosphorus and potassium. Trace elements or micro minerals include molybdenum, fluoride, selenium, zinc, iodine, copper, sulfur, chromium and boron[8]

Macro minerals have different functions in the body. They act together with vitamins and activate the metabolic processes and start the hormone production. Trace elements take part in cellular, tissue and sub cellular functions.[4] The functions include enzyme reaction, muscle contraction, mitochondrial activity, nerve conduction and immune regulation by cellular and humoral mechanism. Micro minerals interact with macro elements and vitamins to increase the body's effective function. So they are important for human health and have different metabolic characteristics and functions[9]

In many studies, the association between trace elements and macro elements with DM has been observed.[4] The action of insulin on decreasing the blood glucose level was potentiated by some trace elements like zinc, manganese, chromium, magnesium, vanadium, selenium and molybdenum[8]

The mechanism by which the trace elements increase the insulin action includes activation of insulin receptor sites, increase in the insulin sensitivity, act as antioxidants, and serve as cofactors in glucose metabolism and prevent lipid per oxidation[9]

Several studies show that the metabolism of several trace and macro elements alters type2 DM and these elements may have some role in the progress and pathogenesis of DM[9] .So here we are trying to know the status of trace elements that have been reported to be involved in glucose homeostasis and their change in levels in type 2 DM.

Classification of diabetes

According to ADA (American Diabetes Association)

1. Type1 Diabetes mellitus (insulin dependent) (IDDM)

- Immune mediated
- Idiopathic

2. Type 2 Diabetes Mellitus (Non- insulin dependent) (NIDDM)

3. Other specific types of diabetes

- Gestational diabetes mellitus (GDM)
- Impaired glucose tolerance (IGT)
- Impaired fasting glucose (IFG)

TYPE 1 DM

5- 10% of the people are with type 1 DM. The abrupt onset of symptoms occurs in type 1 DM. The cause for type 1 DM is

1. Antibodies (as an autoimmune process)
2. Absence of insulin (loss of ß cell and the people need insulin for life long)
3. Type1 idiopathic

Here ketosis is more common. For classification of DM, age is not an important factor.75% of the people will acquire before the age of 18 years.

Increased incidence in childhood and adolescence.Type1 diabetes is otherwise known as auto immune DM[10]

Type2 DM

It is the most common type. 90% of the people are with type2 DM, here ketosis will not occur and the people are not dependent on insulin.

The main mechanism is insulin resistance. It is multifactorial in origin. Obesity is associated with type 2DM, so decreased weight loss will improve hyperglycemia. Mostly it will occur after the age of 40 years. In children and adolescence it is an emerging problem.[11, 12]

Difference between type1 and type2 DM:

FEATURES	TYPE2	TYPE 1
1.Plasma glucose	increased	increased
2. Age of onset	>30 years	<20 years
3.Plasma insulin	normal	absent
4.Body mass	obese	wasted
5.insulin sensitivity	decreased	normal
6.plasma glucagon	High, not able to suppress	High , suppressed
7.treatment	Metformin, insulin, weight loss, sulfonylureas	Insulin

OTHER SPECIFIC TYPES OF DIABETES MELLITUS:

Hyperglycemia is due to

1. Genetic defects of ß cell function
2. Genetic defects in insulin action
3. Disease of exocrine pancreas
4. Endocrinopathies (e.g. cushings syndrome, acromegaly , glucagonoma)
5. Drugs that impair insulin action or decrease the ß cell function (e.g. glucocorticoids , thiazides and ß adrenergic)
6. Infection
7. Immune mediated diabetes and
8. Genetic conditions (e.g. Downs syndrome, klinefelter syndrome , porphyria)

These are termed secondary diabetes.

GESTATIONAL DIABETES MELLITUS (GDM)

"Glucose intolerance with onset or first recognition during pregnancy" [13]

(I.e. women with diabetes, when become pregnant are not included)

Of all the GDM patients,6-62% of them will be are at in the increasing risk for the development of type2 DM. [14]

IMPAIRED GLUCOSE TOLERANCE (IGT)

"People with fasting glucose concentration less than the level for the diagnosis of DM, but glucose value during OGTT is between normal and diabetic state".

2 hrs post glucose load test following OGTT is 140 to 199 mg/dl. Micro vascular disease is rare. The prevalence of mortality from cardiovascular disease and atherosclerosis is increased in IGT patient.[15]

IMPAIRED FASTING GLUCOSE (IFG)

The fasting glucose value is between normal and diabetic state. The value is between 100- 125mg/dl. They increase the risk of diabetes and cardiovascular disease. IFG and IGT are risk factors for cardiovascular disease and diabetes.

CRITERIA FOR DIAGNOSIS

The American diagnostic association for diabetes advises the following criteria for diagnosis of diabetes.

1 "A fasting plasma glucose of ≥126 mg/dl (after no caloric intake for at least 8 hours)" or,

2. "A casual plasma glucose >200 mg/dl (taken at any time of day without regard to time of last meal) with classic diabetes symptoms: increased urination, increased thirst and unexplained weight loss or,

3. ‟ an oral glucose tolerance test (OGTT) (75 gram dose) of >200 mg/dl for the two hour sample. Oral glucose tolerance testing is not necessary if patient has a fasting plasma glucose level of ≥126 mg/dl” or

4. If both the fasting and 2 hr values are above these levels on the same occasion.

5. Glycated hemoglobin level or HbA1c level more than 6.5% at any occasion.

According to American Association of clinical chemistry and American diabetes association, HbA1c is the preferred method for initial diagnosis of diabetes mellitus.

Non-modifiable Risk Factor

- Positive Family History: the incidence increases to 62% if both father and mother are affected by diabetes.
- Genetic studies revealed around 20 genes -like , HHEX, T2CF7L CDKAL1
- FTO, SLC30A8 are having strong relationship with type 2 diabetes

Risk Factors - MODIFIABLE

- waist/hip ratio
- Obesity indicators namely Body mass index(BMI) and
- Waist circumference is also important risk factors for T_2DM.

- Hypercholesterolemia:, Low Density Lipoprotein, elevated levels of total cholesterol, hypertriglyceridemia ,Very Low Density Lipoprotein, and reduced High Density Lipoprotein are risk for type 2 DM. American Diabetes Association says that LDL Cholesterol should be less than 100 mg/dl; HDL Cholesterol must be more than 60 mg/dl and the TGL must be less than 150 mg/dl..
- Hypertensive patients are having double the risk of being diabetics in future.
- The persons : those who are habitually consuming the diets low in whole grains, minerals, antioxidant vitamins decreased intake of dietary fibre nutrients, lignans, and phenol compounds will be having the diabetogenic tendency.

Asians are more prone to develop to type 2DM due to their genetic pattern as compared to West. Asian Indians having more genetic susceptibility and also the environmental factors which makes them to became diabetes. Fatty food along with lack of exercise triggers, environmental and genetic interaction and make them a diabetic.

Pathology of DM

Regulation of blood sugar

During a brief fast, the decline in the glucose concentration is prevented by,

1. Breakdown of glycogen in the liver (glycogenolysis)
2. Synthesis of glucose in the liver (gluconeogenesis) and
3. Also by the gluconeogenesis in kidneys[16]

The skeletal muscle do not participate during the fast brief period because of the lack of the enzyme glucose -6 phosphatase which converts glucose-6 phosphate to glucose [16]

Gluconeogenesis i.e. synthesis of new glucose is the main source when fasting is prolonged for >42 hours. The glucose which is absorbed after a meal is converted to glycogen and stored in the liver and skeletal muscle or as fat in the adipose tissue.

The concentration of blood glucose is maintained within a narrow range by the following hormones[16]

1. Insulin (decrease the blood glucose)
2. Counter – regulatory hormones (glucagon, epinephrine, cortical and GH) which increase the blood glucose

Normal glucose disposal depends on

1. Ability of pancreas to secrete insulin
2. And the ability of insulin to promote uptake of glucose into peripheral tissue and
3. The ability of insulin to suppress hepatic glucose production

INSULIN

Major insulin target organs are

1. Liver
2. Skeletal muscle and
3. Adipose tissue

Insulin is a protein hormone produced by the ß cells of islets of langerhans in the pancreas.

Insulin stimulates the glucose uptake through a specific glucose transporter. GLUT- 4 in to the muscle and fat but not in the liver cells. Insulin is an anabolic hormone.

Functions of Insulin:

1. Uptake of glucose into fat and muscle
2. Promotes the conversion of glucose to glycogen or fat for storage
3. Inhibits glucose production by the liver
4. Stimulates protein synthesis
5. Inhibits protein breakdown

Insulin contains 51 AA and it contains two chain, A and B chain joined by two disulfide bridges, a third disulfide bridge within the A chain.

SYNTHESIS OF INSULIN

Insulin is first synthesized as preproinsulin which is made up of 100 AA formed by the ribosome's in the RER. The preproinsulin is converted to proinsulin by the cleaving enzymes. The newly formed proinsulin is made up

of 86 AA. In the Golgi complex, the newly formed proinsulin is stored in the form of secretary granules. There it is cleaved by the proteolytic enzymes into insulin and connecting peptide (C- peptide) [17]

The synthesis of insulin occurs by two pathways

1. Major pathway
2. Minor pathway

In the major pathway, the proinsulin is cleaved by carboxypeptidase H (CPH) and prohormone convertase 1(PC 1) to form des-31,32 proinsulin. The des 31, 32 proinsulin is converted by CPH and PC2 to c-peptide and insulin.[18]

Minor pathway: The percentage of proinsulin which is metabolized via des 64, 65 proinsulin is < 10%

Insulin release

Insulin release is increased by

1. Amino acids
2. Glucose
3. GI and pancreatic hormones(secretin, gastrin, glucagon and pancreozymin)
4. Drugs like sulfonylureas and ß adrenergic agonists.[19]

Insulin release is decreased by

1. Somatostatin
2. Hypoglycaemia and
3. Drugs like (ά adrenergic agonists, ß adrenergic blockers , phenytoin and nicotinic acid)

There are two phases in the release of insulin.

First phase begins within 1 to 2 min; it causes rapid release of stored insulin. It ends in 10 min .Second phase begins after first phase ends. It continues to release insulin until normoglycemia within 60 to 120 minutes. Normally insulin is secreted in a pulsatile manner, type 1 DM patients has minimal or no insulin response. In type2 DM, the normal pulsatile manner release and the first phase response is lost but the second phase of insulin response is lost.[20]

After insulin is released in to the portal circulation, 50% of insulin is degraded in the liver and also in the kidney. Daily insulin secretion is 1 U / hr and 40 U/day. Half life of insulin is 4-5 minutes.[21]

THE MECHANISM OF INSULIN ACTION

Insulin binds to the insulin receptors located in the plasma membrane. Human insulin receptor is a heterotetramer comprising of 2ά and 2ß subunits. Insulin binds to the ά subunit which is located on the outside of the plasma membrane.[22]

The ß subunit contains an intrinsic tyrosine kinase which extends in to the cell through the plasma membrane. Binding of insulin to its receptor induces a conformational change in the receptor resulting in activation of tyrosine kinase. The tyrosine kinase in turn phosphorylates the tyrosine residues on several proteins. Insulin receptor acts as a substrate for tyrosine kinase. It also phosphorylates various specific intracellular proteins.

The proteins are

1. Insulin receptor substrate proteins (IRS) includes IRS-1, IRS-2, IRS-3, IRS-4
2. Shc
3. Gab-1

These proteins act as deduction site and converts the signal into an electrical one [23]. Transducer protein contains SH2 domain and Src homology 2.

SH2 domain protein[24] contains

1. PI3K (phosphatidyl Inositol 3- kinase)

2. Grb2 (growth factor receptor – bound protein -2)

Both regulate signal transduction events. MAP cascade via RAS was also stimulated by insulin. APKC was activated by PI3 kinase via Akt.APKC regulate glucose transport by modulating translocation of GLUT4. Akt also phosphorylates and inactivates GSK 3 thereby enhancing glycogen synthesis.[25]

TYPE1 DM – PATHOGENESIS

T- Cell mediated autoimmune destruction of the insulin secreting cells of the pancreatic ß cells is the main pathogenesis of the type1 DM. This autoimmune destruction occurs months or years before the clinical presentation. 80 to 90% decrease in volume of ß cells should occur to produce the following symptoms of diabetes.

Markers of autoimmunity are circulating antibodies [26,27,28,29] in the serum before the onset of hyperglycemia. The circulating antibodies include

1. ICAs (islet cell cytoplasm antibodies)
2. IAAs (insulin auto antibodies)
3. Glutamic acid decarboxylase antibodies
4. Insulinoma associated antigens
5. Zinc transporter ZnT8

Before the age of 5, > 90% of the children with type 1 DM has insulin auto antibodies.

Antibodies to GAD occur 10 years before the onset of clinical symptoms. GAD 65 antibodies is seen in patients with type2 DM who will progress to type1 DM.[30, 31]

ZINC TRANSPORTER (ZnT8)

Solute carrier family 30 (zinc transporter) member 8, also known as SLC30A8. It is a human gene that's code for a zinc transporter related to

insulin secretion in humans. Alleles of this gene may increase the risk for developing type2 DM. Loss of function mutation appears to reduce the risk of diabetes.

ZnT8 is the major auto antigen in type1 DM[32]. ZnT8 is linked with 60-80% of the patients with new onset type1 DM and < 3% of with type 2 DM.

Other factors that plays an important part in the pathogenesis of type2 DM are environment and genetics.

GENETICS

Type1 DM is inherited[33] and MHC on chromosome 6 is the site. HLA – DQ and DR genetic factors are involve d in type1 DM.[34] 95% of type1 express HLA-DR3 or HLA-DR4. The risk of diabetes can be identified by HLA typing.[35] 10 % of the patients with type1 DM have an affected first degree relative.

ENVIRONMENTAL FACTORS

Viruses such as Coxsackie virus B, rubella and mumps are important environmental factors in the pathogenesis of type1 DM[36.] These viruses will cause autoimmunity to ß cells by producing viral protein or some other environmental insult.

TYPE 2 DM- PATHOGENESIS

The pathology behind the type 2 DM [37, 38, 39] are

1. ß -cell dysfunction (the pancreas not able to secrete insulin to balance for insulin resistance) (Holt 2004)[40]
2. Insulin resistance leads to impaired insulin action

Insulin resistance is the primary defect preceding the derangement in insulin secretion[38, 39].Type 2 DM is a heterogeneous disease .There is no single cause for type2 DM. A combination of genetic, environmental and molecular defects plays a role in the pathogenesis of type2 DM.

Prevalence is much greater in developing than in developed countries (Shaw et al 2010).[41]

People with the age group 40- 60 years are most affected. People with more than 60 years are affected most in the developed countries. (Shaw et al, 2010)[41]. The cause for increase in type2 DM is mainly due to

1. Changes towards a western life style in developing countries[42] (high diet with reduced physical activity)
2. Increase in prevalence of overweight and obesity[43]

The life time risk of developing diabetes 1 in 10 (Neil et al 1987)[44]. Type2 DM increases with increase in age and also more after the age of 40 years.

The overall life time risk of developing DM is 1 in 10 (Neil et al).[44] The environmental factors include stress, aging, obesity , lack of exercise and overeating[45](Kaku 2010). Type 2DM is also known as Idiopathic diabetes. The genetic susceptibility for type2 DM is not known. Thus we conclude that type2 DM may be due to heterogeneity of the genes. Compared to type1 DM , type2 DM has increased genetic predisposition[40].

LOSS OF ß CELL FUNCTION

The fasting hyperglycemia occurs due to insulin resistance which increases the ß - cell demand , which inturn leasd to progressive ß- cell function loss.

"Selective glucose unresponsiveness" is the term given to loss of glucose induced insulin release. The term glucotoxicity is given to hyperglycemia causing the ß - cells unrespo nsive to glucose.the level of ß-cell dysfunction correlates with both the glucose concentration and duration of hyperglycemia. Increased fatty acid synthesis will also lead to ß- cell failure.[46] When the blood glucose level returns to normal the defect will resolve.

OTHER ABNORMALITY IN TYPE2 DM

1. Increased ratio of plasma proinsulin to insulin[47]
2. Decreased normal pulsatile release of insulin

INSULIN RESISTANCE

It is defined as "decreased biological response to normal concentration of circulating insulin" [48].it is seen in both type2 DM patients and also in obese non diabetic individuals.

The causes of insulin resistance are given below.

1. Excess growth hormone (acromegaly)
2. Obesity / over weight (especially excess visceral adiposity)
3. Pregnancy ; gestational diabetes
4. Lipodystrophy (acquired or genetic, associated with lipid accumulation in the liver.
5. polycystic ovary disease
6. excess glucocorticoids (cushings syndrome or steroid therapy)
7. An inherited disease that causes iron accumulation in tissue (Hemochromatosis)
8. mutations of insulin receptor
9. Auto antibodies to the insulin receptor
10. mutations that cause genetic obesity(eg melanocortin receptor mutations)
11. mutations of the peroxisome proliferators activator receptor γ (PPARγ)
12. Insulin resistance is not known, but may be due to defect in

13. Insulin action.

14. Decreased insulin receptor number

15. Secondary to hyperinsulinemia and hyperglycemia or

16. Due to decreased tyrosine kinase activity.[49, 50,51] (Comi et al, 1987, Bonadonna et al, 1993, Sten- lindes et al, 1993)

The incidence of type2 DM is 1 to2% in Caucasians, [52](in1993 by Cook et al), and increased in some ethnic groups. They are Arabs (Richens et al 1988) in south India [53]and Pima Indians(Knowler et al 1990). Here the prevalence is about 50%.[54]The mode of inheritance in type2 DM is not clear except MODY.

MODY (Maturity onset diabetes of the young)

It is inherited as an autosomal dominant trait. The mutations are at chromosome 7P and the gene is the Glucokinase.[55, 56] (Froguel et al, 1993: Hattersley et al, 1992) It is more common in blacks and Indians. It occurs after the age of 25 years and it is treated for more than 5years, without insulin .they are mainly Islet cell cytoplasmic antibodies negative.

One of the important cause for multifactorial disease is that the association of the polymorphic 5 ' flanking region of the human insulin gene and type 2 DM is lacking in some population groups.

The defects in insulin secretion may be due to

1. Increased sensitivity of AA to insulin release.

2. Decreased first and second phase of insulin response

3. Relative decrease in basal insulin secretion and

4. Glucose insensitivity.

MEASUREMENT OF INSULIN RESISTANCE

1. Direct – insulin concentration in the fasting state

2. Euglycemic insulin clamp [57] by indirect method and

3. HOMA – Insulin resistance

Two types of clamps. Hyper glycemic clamp and euglycemic clamp. Hyperglycemic clamp – it maintains high blood sugar level by infusion or perfusion with glucose. Used to quantify how fast ß - cell respond to glucose. Euglycemic clamp – it shows how sensitive is the tissue to insulin. In this normal blood sugar level is maintained.

SYNDROME X

The other name for syndrome x is metabolic syndrome or insulin resistance syndrome. It consists of clinical and laboratory findings[58].

1. Hyperinsulinemia
2. Insulin resistance

3. Hypertension
4. Obesity and
5. Dyslipidemia(decreases TGL and decreases HDL cholesterol)

The diagnosis of syndrome X is attained if a person has 3 or > 3 of the given criteria below[59],

1. FPG > or equal to 110 mg/dl
2. TGL > 150mg/dl
3. Abdominal obesity i.e. waist circumference > 35 inches in women or 40 inches in men
4. Blood pressure > or equal to 130/85 mmHg
5. HDL cholesterol < 50 mg/dl in women and < 40mg/dl in men

People with this syndrome are at increased risk for cardiovascular disease.

ENVIRONMENTAL FACTORS

The environmental factors include diet and sedentary life style. Lack of exercises are important determinant in the pathogenesis of type2 DM. there is a close relationship between obesity and type 2 DM. Of all the obese individual, only 15% of them will develop DM. But they are all having hyperinsulinemia and are insulin resistant. Exercise increases the sensitivity to insulin in adipose tissue and skeletal muscle.

OTHER FACTORS INCLUDE

1. Duration of obesity
2. The Distribution of fat and
3. The genetic predisposition (family history of type2 DM)

Obesity - when the BMI > than or equal to 30 kg/ m^2 leads to increase in prevalence of type2 DM. [60,61]Many randomized controlled trial studies showed that changes in life style like exercise and weight reduction decreases the incidence of type 2 DM.

VISCERAL ADIPOSITY

The causes for increase in visceral fat (Kaku 2010)[62] are

1. Smoking
2. Genetic factors
3. Increase in alcohol intake
4. Aging
5. Over eating , especially excessive intake of simple sugars
6. Stress - related factors
7. Decreased energy is utilised due to absence of exercise
8. Disorders of endocrine and nervous system – there is increase in cortisol secretion and sex hormone secretion.

DIABETOGENES

The genetic factors contribute to type 2 DM[38], but the mode of inheritance is not known. Type 2 DM is also known as " geneticist's night mare"[63.]The search for diabetogenes in type 2 DM is complicated by multiple factors. It is more common in fatty person whose parents are diabetic than in fatty person with no history of diabetes in the family.

- 60 genes are associated with type2 DM. But only 5% of the people with type2 DM have genetic defects. So the genes carrying type2 DM remains unknown. Only the genes involved in insulin secretion, insulin action and regulation of body weight are known.

CANDIDATE INSULIN SECRETION GENES

Genes that are expressed in ß-cell are Amylin, glucagon – like peptide-1 receptor, glucokinase regulatory protein and Islet-1 protein**.**

INSULIN RESISTANCE GENES

Several mutations of the insulin receptor genes (INSR) have been identified[48]. But these mutations are rare. There are few other mutations that code for GLUT4 and glycogen synthase. Genetic variation in the gene encoding calpain -10 appears to increase the diabetes susceptibility in selected population.

CANDIDATE BODY WEIGHT GENE

The gene in adipose tissue is the ob gene. The ob gene is cloned and its protein product Leptin is important in regulating body weight homeostasis and energy balance. Plasma Leptin concentrations are increased in diabetics.

OTHER FACTORS

AMYLIN: (Islet amyloid polypeptide IAPP)

It is 37-AA peptide, stored in the ß- cells of pancreas. It is secreted along with insulin in response to food ingestion. > 90% of the people with type2DM have amyloid deposits in pancreatic islet. The effect of Amylin in the causation of type2 DM is not known. But there are many studies that show that excess Amylin causes insulin resistance[64] and glucose intolerance in type2 DM.

PATHOPHYSIOLOGY OF TYPE2 DM

Patients with type2 DM have some detectable levels of circulating insulin compared to type1 DM patients. On the basis of oral glucose tolerance test, people with NIDDM can be divided into four groups.

1. DM with minimal fasting hyperglycemia (fasting plasma glucose < 140mg/dl)
2. Those with normal glucose tolerance
3. Impaired glucose tolerance and
4. Diabetes mellitus in association with overt fasting hyperglycemia(fasting plasma glucose > 140 mg/dl

Individuals with impaired glucose tolerance (IGT) have hyperglycemia, secreting normal insulin level but them are resistant to action. From conversion of IGT to DM, the insulin level declines indicating that the NIDDM patients have reduced insulin discharge. In type2 DM both decreased insulin secretion and insulin resistance are common.[40] (Holt, 2004). The most important cause for type2 DM is insulin resistance. In 2010 according to Raju and Raju reduced insulin secretion is the first cause because IR alone is not enough to cause type2 DM.[65] (In 2010 by Raju and Raju).

Both the defects will be seen in most of the patients. A member of the nuclear hormone receptor protein super family is a recent concept for the cause of type2 DM[65] (In 2010 by Raju and Raju).

Thiazolidinedione are the latest group of medicines which increases the body to insulin sensitivity. The function of peroxisome proliferators activation reactors g(PPARg)is altered by these medicines. PPARg is the replication factor. It gets activated and it binds to another replication factor called the RXR (Retinoid X receptor). PPARg is the controller of fat tissue differentiation. It causes the separated and un separated cells into mature adipose tissue cell. It is known for the making of important substances from the vascular endothelial cells and from the immune cells.[65] (Raju and Raju, 2010)

PATHOGENESIS OF CHRONIC COMPLICATIONS OF DIABETES MELLITUS

The complications are divided into

1. Micro vascular complications
2. Macro vascular complications

Micro vascular complications include peripheral nerve, retina and renal glomerulus causing neuropathy, retinopathy and nephropathy[66]. DM is one of the most common causes for end stage renal disease[67]. It is the main cause for newly diagnosed cases of blindness. Atherosclerosis is the macro vascular complication involving peripheral large vessels, cerebral and cardiac vessels[68, 69]. The most common cause for mortality in diabetic patients is due to increasing rate of Myocardial infarction in these patients. Not only Myocardial infarction but also have increased rate of limb amputation and stroke.

There is an association between increased blood glucose level and the progression of micro vascular complications.[66]. The important factor contributing to the pathogenesis of macro vascular complications include insulin resistance and hyperglycemia.[67, 68, 69]

The hypothesis was put forth to know how increase in blood glucose level will lead to vascular and neural complications. They are

1. Increased formation of advanced glycation end products (AGE)
2. Increased hexosamine pathway flux
3. Polyol pathway or increased aldose reductase flux and
4. Activation of protein kinase c

COMPLICATIONS OF DIABETES MELLITUS

ACUTE	CHRONIC
Diabetic ketoacidosis Hyperglycemic hyperosmolar state Hypoglycemia Diabetic foot ulcer Infections	**MICROVASCULAR** Retinopathy Nephropathy neuropathy **MACROVASCULAR** Accelerated arteriosclerosis Myocardial infarction Stroke

The importance of trace elements gaining attention for the past 30 yrs .Since trace elements are playing vital role in many metabolic process and they are needed for growth and immunological functions , they behave as catalyst and interact with enzymes and biological membranes and modify their actions. Zinc has action over tissues like enterocytes, pancreas and hepatocytes. In type 1dm persons the levels of zinc seems to be reduced .[4]Zinc and magnesium serves as a cofactor for several tissue functions, synthesis, storage and release of insulin. Zinc stimulates the insulin secretion. The zinc deficiency may possibly exacerbate the insulin resistance in type1 diabetes mellitus and NIDDM. The dietary zinc scarcity, along with zinc insufficiency in diabetic persons, will lead to the pathogenesis of type 2 diabetes. Studies

have showed there is an increase in response to insulin sensitivity after the supplementation of zinc. .Magnesium has a pivotal role in glucose homeostasis at various portions of metabolism. A compound arrangement is maintained between glucose metabolism and Magnesium. Hypomagnesaemia is seen in NIDDM and IDDM. Magnesium acts as a coenzyme for more than three hundred enzymes, including the enzymes of glucose oxidation, and release of insulin. Magnesium and its intracellular levels accelerate the insulin secretions. Till the physiology has to be understood.

ZINC IN HUMAN METABOLISM

Zinc is an important trace element. It act as coenzyme for more than 300 enzymes which are taking part in the various metabolisms like carbohydrate, proteins, lipids and Nucleotide .zinc is present in skeletal muscle, bone and plasma membrane. High zinc content is present in choroid plexuses of the eye and in prostatic fluid [70] . The molecular integrity of cell membrane is maintained by the zinc and also the physiological integrity of many organs like pancreas, choroid and the intestine. It controls the expression of genes. Another important function of zinc is in the immune system by Shanglar et al[71]

Clinical features of zinc deficiency include diarrhoea, alopecia, and increased susceptibility to infections, impaired appetite and behavioural changes[70]. Mild zinc deficiency changes are less clear. Low zinc intake results in impaired taste and wound healing and these effects are less observed.

Metabolism of zinc

Zinc absorption is concentration dependent and its absorption occurs throughout the intestine. Zinc absorption occurs fast in aqueous solution, but its absorption is less in solid diets.[72]

Zinc is excreted through the skin, intestine and kidneys. The excretion of zinc in urine occurs during starvation and muscle catabolism. Lost through skin occurs during strenuous exercise and elevated ambient temperatures.[73]

No zinc stores in our body. Zinc is released from the bone resorption and tissue catabolism and it is reutilised. Many studies have shown that the enzymes which contain zinc and its level in the blood will maintain the normal zinc level for many months which shows that how zinc is balanced in our body. The mechanism of zinc homeostasis is poorly understood.[74-77]

Many physiological indexes of zinc status have recommended, that, taste acuity, dark adaptation and wound healing needs zinc.[78] These indexes are not for identifying zinc deficiency and also not particular to zinc.

ZINC DEFICIENCY- MINIMAL

The radioisotope technique in the investigation of zinc [79] produced possibilities of the association involving diet and zinc and the significance in accepting the role of zinc in the homeostatic regulations .The radioisotope relinquish in plasma and they exposed the body zinc with a reference range of

100-200 mg [80-83].The amount of zinc in the body relates to the regular intake and it is decreased in controlled depletion researches .[82]. The zinc store in the body is related to the body defecation of zinc and to the total assimilation of zinc. The zinc store depends on the absorbed zinc and the body store ends in defecation. For the preservation of zinc, the defecation of zinc is most significant than the absorptive changes.[83]

SOURCE OF ZINC

The richest sources of zinc are red meat, whole grain cereals, pulses and legumes, The concentration in these sources is 380-760 mmol/Kg (25-50mg/kg) dry weight. Moderate sources of zinc are meat with high fat, polished rice and lean meat. They have concentration of about 10-25 mg/kg (150-380 mmol/kg). The least sources of zinc are spinach, fish, fruits and roots and they contain about <10 mg/kg(<150 mmol/kg). Detached oils and fats, alcohol and sugars having a very minimal zinc content.[84]

The use of zinc in our body depends on the overall composition of the diet. Many New studies have recognized that, many food factors act as promoters or antagonists of zinc absorption[85.] Amino and hydroxy acids, the soluble organic compounds with low molecular weight facilitate zinc absorption. The zinc absorption is impaired by the organic compounds that are forming poorly soluble complexes with zinc. [85]

Researches with Radio isotope studies in humans have recognized that the major factor for zinc utilisation and absorption is the total body zinc content, the phytate content and the dietary protein. The amount of phytate is smaller in vegetables and high in legumes and whole grain cereals. When phytate bind with divalent Cation, it reduces the zinc digestion and that has been shown in human beings [85.] The molar ratio between phytate and zinc in food is an effective indicator of phytate level in reducing zinc digestion.[84]

The effect of phytate is decreased by the amount of protein in the diet. Calcium in the diet increases the antagonistic effect of phytate .and these depend on source of calcium and the composition of diet. [86]

Several studies showed that zinc is absorbed twice as much from meat than wheat and rice. The details of zinc absorption from diet in developing countries, which have high phytate content, are not known .So decrease in phytate content of the diet and inclusion of animal protein will improve the zinc absorption.[84, 87, 88].

To reduce the phytate content we have to activate phytase or by the addition of microbial or fungal phytases. The action of phytases is to hydrolyse it into Inositol phosphates, which results in increased zinc absorption. [89, 90]. The phytase activity is increased by germination of cereals and legumes

RISK GROUP FOR ZINC DEFICIENCY

1. CHILDREN

The deficiency of zinc leads to stunting in children which was proved in several studies [91] and increase in weight gain in children when supplemented with zinc those who are malnourished. "A recent meta-analysis of 25 intervention trials comprising 1834 children under 13 years of age, with a mean duration of approximately 7 months and a mean dose of zinc of 14 mg/day (214 mmol/day), showed a small but significant positive effect of zinc supplementation on height and weight increases [92]."

The studies on zinc supplementation results show that zinc deficiency not only reduces the growth of the chid but also leads to severe infectious diseases. [93] The decrease in frequency of acute diarrhoea has also been reported in many studies. Zinc supplementation reduces the incidence of malaria and LRTI (lower respiratory tract infection) and it was shown in various researches. So the child growth and health can be improved by zinc supplementation.

2. PREGNANCY

Maternal zinc and its effect on pregnancy are not known. Negative as well as positive correlation has been reported between serum zinc concentration and foetal growth or labour and delivery complications[94]. In pregnancy because of hemodilution plasma zinc levels will be low. The study in American women with low income group whose plasma zinc concentrations were below the

mean at the time of antenatal period showed that intake of 25mg/day of zinc results in greater infant birth weights and head circumferences compared with the controls[95].

THE REQUIREMENT OF ZINC

The WHO report in 1996[96] showed that zinc requirement was calculated by a special technique in which requirement for growth of the tissue , internal losses and metabolism are calculated by adding. Our body has the capacity to adjust to different levels of zinc intake by internal zinc losses through the skin, intestine and kidney.[97] The requirement of zinc absorption is defined as the loss of zinc during the first phase of zinc reduction before the adaptive decrease in zinc excretion. For men and women the requirement of zinc absorption was set at 1.4 mg/day and 1.0 mg/day . The physiological requirements during the gestational period and breast feeding is the retention of zinc during these periods[96].

The estimates of absorbed zinc to requirements involve several considerations. First nature of the diet that determine the zinc absorption, second the effect of absorption is inversely related to the zinc content in diet. The study on zinc absorption results showed that there are three categories 1.high 2. Moderate 3.low zinc bioavailability as shown in Table [96] below. The availability of zinc was used to categorise the diet.

GROUPS	MAIN DIETARY CHARACTERISTICS
High availability	Diets with decreased fibre content, decrease in phytate content . The molar ratio between zinc and phytate is < 5, non-vegetarian diet such as meats, fish
Moderate availability	Mixed diet containing animal or fish protein. Phytate – zinc (molar) ratio within 5-15 or not exceeding 10.The zinc content in the diet increases when it includes protein sources or animal diet.
Low availability	Diet high in ungerminated, unrefined and unfermented cereal grain when added with inorganic calcium salts and intake of animal protein is not sufficient. The molar ratio between zinc and phytate exceeds 15.

The capacity of zinc digestion and its content are different for these diets. Table shows the average individual dietary zinc requirement. The absorption percentage for 3 diet categories were high bioavailability – 50%, moderate bioavailability- 30% and low bioavailability – 15%.

From the data of dietary intake studies, mean population intakes were identified, which shows that low prevalence of individuals at risk of inadequate zinc intake[96]

CHILDREN, ADOLESCENTS AND INFANTS

The requirement of zinc for 3 months old infant for both male and female

were 120 – 140 µg/kg/day. For age 6-12 months the value decreased to about 33µg/kg/day. For age 1- 10 years it is 30µg/g. At puberty the physiologic zinc requirement increases. For adolescent male the zinc requirement is 0.5µg/g [97]

PREGNANCY:

The physiologic requirement of zinc during third trimester is twice as high as that in nonpregnant women.[96]

LACTATION:

Human milk contains high zinc concentration of 2- 3 mg/L in the first month. After 3 months it fall to 0.9mg/L. Daily output of zinc in milk during the first 3 months of lactation is about 1.4mg/day.[98]

ELDERLY:

The requirement of zinc for the elderly is same as that for adults. But the absorption of zinc is low in the elderly, so the dietary requirement of zinc is high. Endogenous los ses of zinc in the elderly is low.

VARIATIONS IN ZINC REQUIREMENT BETWEEN THE PERSON AND THE INTAKE OF NUTRIENT REQUIREMENT

The physiologic requirement of zinc is the same as protein requirement because the requirement of zinc also depends upon the tissue growth and tissue destruction.[99] For the calculation of zinc absorption between individual

variation its absorptive efficacy should be taken into account. There are only few studies on the variation of zinc absorption between persons.. In another study with few groups, the reported variations in zinc absorption are independent of age, sex or diet. Data available [83,84,87-90,100] from the zinc absorption studies shows that variations in dietary zinc requirements includes variations in requirements for absorbed zinc and also includes variations in absorptive efficiency corresponds to a CV of 25%.

AVERAGE INDIVIDUAL NORMATIVE REQUIREMENTS FOR ZINC FROM THE DIETS DIFFERING IN ZINC BIOAVAILABILITY (µg/kg body weight/day)

Age in years	**High bioavailability**	**Moderate availability**	**Low bioavailability**
	µg/kg body weight/day		
Infants and children			
1-3	138	230	459
3-6	114	190	380
6-10	90	149	299
Adolescents			
males 15-18	61	202	205
Females15-18	56	73	187
Adults			
Females 18-60+	36	59	119
Males 18-60+	43	72	144

Upper limits of zinc intake:

Increased zinc intake for long time affects the metabolism of other trace elements. zinc intake of > 50mg/day affects the cuzn- superoxide dismutase in erythrocytes.[99,101]copper is very sensitive to increased zinc intake. when zinc intake occurs over 450- 660mg/day [102,103],the copper and ceruloplasmin levels is decreased which lead to anaemia.

The upper limit of zinc for adult men is 45mg/day and for children is 23-28mg/day. Zinc toxicity occurs due to excessive intake of sea foods and from galvanized cooking utensils. Recommended Nutrient intakes (RNIs) for dietary zinc (mg/day) from diets differing in zinc bioavailability:

Age group	Bio high availability	Bio-moderate availability	Bio- low availability
Infants and children			
0-6 months	1.1	2.8	6.6
1-3 years	2.1	4.1	8.3
Adolescents Female (10-18 yrs)	4.3	7.2	14.4
Male (10- 18yrs)	5.1	8.6	17.1
Adults			
Females 19-65yrs	3.0	4.9	9.8
Males (19-65yrs)	4.2	7.0	14.0
Pregnant woman			
I trimester	3.4	5.5	11.0
II trimester	4.2	7.0	14.0

III trimester	6.0	10.0	20.0
Lactating women			
0-3 months	5.8	9.5	19.0
3-6 months	5.3	8.8	17.5
6-12 months	4.3	7.2	14.4

GLYCATED PROTEINS:

Glycated haemoglobin (GHb) is a retrospective index. In diabetic people it is an effective monitoring of long term glucose control. In addition to blood glucose estimation, it is also used to monitor long term glucose control. It has been recently advised for diabetes diagnosis. It is also used to calculate the risk of development of micro vascular complications.

GLYCATED HEMOGLOBIN:

Glycation is the " non-enzymatic addition of a sugar residue to amino groups of proteins"

Several minor hemoglobin's like HbA1a, HbA1b, and HbA1c together constitute HbA1. HbA1a has two subdivision i.e. $HbA1a_{1,}$ $HbA1a_{2.}$ This minor hemoglobin's otherwise called as fast hemoglobin's because they travel more quickly than HbA in an electric field.

The carbohydrate residues attached to the several minor hemoglobin's are,

HbA1a

- $1a_1$ – HbA + fructose 1,6 diphosphate
- to the N- terminal of the ß chain
- $1a_2$- HbA + glu 6-phosphate attached to the N- Terminal of the ß chain.

HbA1b – HbA + Pyruvic acid attached to the N-terminal of ß chain

HbA1c – HbA + glucose attached to the N-terminal Valine of the ß chain.

HbA1c is formed by the condensation of glucose with the N-terminal Valine residue of each ß chain of HbA to form an unstable Schiff base (Aldimine) or preHbA1c.

Schiff base may undergo Amadori rearrangement to form a stable ketoamine, HbA1c.

HbA1c is the major fraction, constituting 80% of HbA1. Fast hemoglobin HbA1 is also called as neoglycoprotein. Glycation other than the ß chain such as lysine residues or ά chain are called as glycated hemoglobin HbA_0 or " total glycated hemoglobin". It is measured by Boronate affinity chromatography.

The formation of glycated hemoglobin is irreversible. Concentration in blood depends on the life span of the RBC and blood glucose concentration.

"GHb concentration represents the integrated values for glucose over the preceding 8 to 12 weeks". It is unaffected by the day to day glucose values and food ingestion or exercise. The change in the level of HbA1c is fast during the first two months of blood glucose alteration and achieves a steady state 3 months later.

Decrease in glycated hemoglobin:

1. Haemolytic disease[104]

2. Recent blood loss (due to increase in young RBC

Increase in GHb:

Iron deficiency anemia (because of increase in old RBCs)

Interferences in GHb determination:

1. Haemoglobin variants (Hb F , HbC , HbS)[104]

2. Carbamylated haemoglobin

(Attachment of urea to HbA in renal failure patients. Common in diabetics)

3. Schiff base (Pre HbA1c)

The Schiff base (unstable fraction) is not an index of long term glucose control because it changes with change in the concentration of blood glucose. These Schiff base constitute (Pre HbA1c) 5-8% of total HbA1[105] . In the absence of glucose, preHbA1c converted to glucose and HbA. While determination of HbA1c, pre HbA1c should be removed first. To remove this

labile fraction, incubate the washed red blood cells in saline. In Boronate affinity there is rapid dissociation of Schiff base.

"A major change in the diagnosis of diabetes was recommended in 2009"[106,107].

"HbA1c could be used for the diagnosis of diabetes" by the international expert committee. It was also recommended by both ADA (American Diabetes Association) and WHO. [108] The decision point is ≥ 6.5% based on the prevalence of retinopathy[107].

HbA1c level between 5.7 to 6.4% - high risk for developing diabetes.

"ADA recommends that HbA1c as an alternative to glucose for screening for diabetes."[108]

The average HbA1c concentrations were symmetrically related to the risk of retinopathy and nephropathy. 10% reduction in glycated hemoglobin concentration will reduce the risk of retinopathy by 45%. [109] In patients without diabetes, HbA1c is directly related to cardiovascular disease.

Increase in 1% of HbA1c will increase the risk of death by 28%.[110]

Each 1% decrease in HbA1C from 8% to 7%, will reduce the risk of micro vascular complications by 37%, 21% of death and 14% of MI [67] . So diabetes patients should have HbA1c between less than 6.5 to 7 %.[111]

METHODS FOR THE DETERMINATION OF GLYCATED HEMOGLOBIN (GHb)

Various methods are available. They are

1. Based on charge differences
    a. Ion exchange chromatography
    b. HPLC
    c. Electrophoresis
    d. Isoelectric focusing
2. Separated based on structural differences
    a. Affinity chromatography
    b. Immunoassay
3. Based on the chemical analysis
    a. Photometry
    b. Spectrophotometry

Result is expressed as % of total hemoglobin.

MAGNESIUM

Next to potassium, magnesium is the second most common intracellular cation. It is the fourth most abundant cation in the body[112]. Of the total magnesium content 55% in skeleton and 45% is intracellular.

Hypomagnesaemia is more common in hospitalized patients with a prevalence of about 10%. Magnesium plays many physiological roles in the body. These include

1. Membrane function
    - Transmembrane electrolyte flux
    - Cell adhesion
2. Structural function
    - Protein
    - Mitochondria
    - Nucleic acids
3. Enzyme function

ATP ases or GTP ases	-	Na^+ K^+ - ATP ase
		Ca^+ ATP ase
Cyclases	-	Adenylate Cyclases
		Guanylate Cyclases

4. Enzyme substrate kinases B –
    - Hexokinase
    - Creatine kinase
    - Protein kinase

    Direct enzyme activation
    - Phosphofructokinase
    - Adenylate kinase
    - Creatine kinase
5. Calcium antagonist
    - Neurotransmitter release
    - Muscle contraction / relaxation

More than 300 enzymes are dependent on magnesium[113]. It is essential for the synthesis of nucleic acids and proteins[114]. It is also needed for the intermediary metabolism, neuromuscular and cardiovascular systems.

MAGNESIUM METABOLISM

Normal adult human body contains 1000 mmol of magnesium[112] (22- 26 g)

Distribution of magnesium in adult human is shown in the table below

Tissue	% of total body magnesium
Serum	0.3
RBC	0.5
3 .Soft tissue	19.3
Muscle	27.0
Bone	52.9

Of the 60% of magnesium in bone, 30% is exchangeable and function as a reservoir to stabilize the serum concentration.

Normal adult total Serum Magnesium is between 0.70- 1.10 mmol/L(1.7-2.4 mg/dl). There are three forms of magnesium similar to calcium. They are protein bound 20%, free form (ionized) 65% and 15% mingled with negatively charged ions such as citrate and phosphate. Of the three forms of magnesium in plasma only the ionized magnesium has the greatest biological activity.

Serum ionized magnesium concentration is 0.54- 0.67 mmol/ L is limited than that for calcium. Of the total cellular magnesium, free ionized form is only 0.5-5%. The remaining is linked to the negatively charged compounds such as ADP, Citrate, RNA, ATP, Proteins and DNA or is destroyed within mitochondria and Endoplasmic reticulum.

MAGNESIUM- SOURCE

It depends on the magnesium concentration in drinking water and food composition.

Magnesium is rich in cereals, green leafy vegetables, nuts, grains and legumes. Fruits, chocolates, vegetables, meats and fish have intermediate values and dairy products are poor in magnesium

RDA for magnesium in adults is 4.5mg/kg/day[112]. The requirement is high in pregnancy, lactation and following debilitating illness. The Important source of magnesium is drinking water especially " Hard water", which contains up to 30mg/L of magnesium. Cooking results in significant loss of magnesium rich foods.[115]

ABSORPTION OF MAGNESIUM:

Average magnesium intake of normal adult is 12mmol/day.2 mmol/day is secreted in to the intestinal tract in pancreatic, bile and intestinal juices. From this 6 mmol (30%) is absorbed giving a net absorption of 4 mmol/day.

It is mainly absorbed in the ileum and colon. The process of absorption is passive. PTH is the hormone regulating the magnesium absorption. Its absorption is impaired by the phytates in the diet. Its absorption is also affected by the protein, potassium, phosphate, oxalate and zinc. Absorption is not directly proportional to magnesium intake but is dependent on magnesium status. Lower the magnesium level; the more is absorbed in the gut and vice versa.

MAGNESIUM HOMEOSTASIS:

The major organ which maintains the magnesium homeostasis is kidney. Of the 80% of ultra filtrate, 84 mmol of magnesium is filtered daily and 95% of it is reabsorbed. Only 3-5 mmol appears in the urine. In the proximal convoluted tubule (PCT) 15 -20% of the filtered magnesium is reabsorbed. 65-75% is reabsorbed in the thin ascending loop of Henle (TALH) and the rest in distal segments[115, 116]. Magnesium reabsorption mainly depends on the magnesium levels in plasma. Conversion factor from mg to mmol is 0.04113. Magnesium excretion occurs in circadian rhythm. Maximum excretion occurs at night.

FACTORS AFFECTING THE TUBULAR REABSORPTION OF MAGNESIUM

1) GFR
2) Hormones (PTH, calcitonin, insulin, ADH and glucagon)
3) volume status
4) Plasma magnesium concentration
5) phosphate depletion and
6) Hypercalcemia

Plasma magnesium concentration is the major factor in urinary magnesium excretion. When dietary intake of magnesium is decreased, fractional excretion is decreased to < 0.5% due to increased reabsorption in TALH. [114]

MAGNESIUM AND DIABETES MELLITUS:

Magnesium deficiency is more common in both type 1 and type 2 DM[117]. It accounts for 25-39% of patients. The cause for hypomagnesaemia in both types of DM is due to increased magnesium excretion caused by osmotic dieresis and also due to tubular defect[118]. The decrease in serum magnesium level is interrelated with fasting blood glucose, duration of diabetes and glycated hemoglobin[119]. The pathogenesis behind the development of diabetic complications is magnesium depletion and its effect on Inositol transport[120].

MAGNESIUM AND GLUCOSE HOMEOSTASIS:

Since magnesium is the cofactor for many enzymes involved in glucose metabolism, its deficiency will alter the glucose homeostasis [121]. It affects the carbohydrate metabolism by influencing the insulin secretion and glucose entry in to the cells. Its inadequate intake will inhibit the release of insulin in response to a glucose load.

Magnesium increases the insulin sensitivity and its deficiency will lead to insulin resistance[121]. Conversely insulin resistance will lead to a lower serum magnesium concentration. So magnesium supplementation improves the glucose disposal in diabetic patients and decreases.

MAGNESIUM DEFICIENCY AND ATHEROSCLEROSIS:

Various empirical studies showed that inadequate magnesium intake may play an important part in the pathogenesis of atherosclerosis [122]. Magnesium deficiency is characterized by increased cholesterol, VLDL, LDL TGL and decreased HDL, apoA_1 and plasma LCAT activity[123].

Increased lipid per oxidation due to the production of free radical and increased platelet collection may lead to atherosclerosis.

When magnesium is supplied to those patients there is a decrease in total and LDL cholesterol and increase in HDL cholesterol[123]. There is a inverse relation between magnesium intake and blood pressure[121]. The vascular tone is increased due to magnesium deficiency[124].

ASSESSMENT OF MAGNESIUM STATUS:

Three major approaches for magnesium testing ,

1. Magnesium in serum, RBC, leukocytes and muscle
2. Metabolic assessment via isotopic analysis, renal excretion of magnesium
3. Free magnesium levels with ISE (ion selective electrodes), NMR spectroscopy (nuclear magnetic resonance) and fluorescent probes.

Total magnesium content of a sample can be determined by using flame AAS. For intracellular free magnesium concentration, NMR may be used[112].

The reference method for serum magnesium estimation is AAS (atomic absorption spectrophotometer). Serum magnesium concentration does not reflect the total body magnesium levels.[125,126]

Some people with chronic magnesium deficiency has normal serum magnesium level but still have a deficit in total body magnesium and vice versa[125]. Magnesium concentration in RBCS is higher than in serum [127,128](1.65-2.65 mmol/L) .Today serum magnesium estimation by photometric method become the routine practice in most of the laboratories. Magnesium load test or retention test is used to identify hypomagnesaemia. But it is not a routine practice.

HYPOMAGNESEMIA:

Hypomagnesaemia is when the serum magnesium level is < 1.5mg/dl[129,130]. It is more common in hospitalized patients. Prevalence is 9 to 65%[129,130].

High incidence in ICU patients.

CAUSES FOR HYPOMAGNESEMIA:

1) Gastrointestinal disorders:
   - Acute and chronic diarrhoea
   - Renal loss
2) osmotic dieresis
   - Glucose (diabetes mellitus)
   - Mannitol
   - Urea

3) Hypercalcemia

4) Alcohol

5) Malabsorption syndrome

6) Metabolic acidosis (starvation, ketoacidosis, alcoholism)

7) Renal diseases(RTA, chronic pyelonephritis, glomerulonephritis)

8) Drugs[129] (diuretics, amino glycosides, cisplatin , amphotericin B and cyclosporine)

Clinical signs of hypo and hypermagnesaemia overlap. The early signs are nausea, vomiting, fatigue and weakness[129]. Other signs include tremor, depression, hypokalemia, cardiac arrhythmia, agitation and muscle fasciculation.[129]

HYPERMAGNESEMIA

CAUSES

1. Excessive intake of antacids, enema and treatment of pregnancy induced hypertension.
2. Chronic renal failure
3. Acute rhabdomyolysis and
4. Lithium ingestion

It is also more common in hospitalized patients. Its prevalence is 5.7 to7.9%[129].

Clinical signs

Hypotension,nausea,vomiting,neuromusculardysfunction,hypotonic areflexia. ECG findings include prolonged QT interval, PR and QRS interval, complete heart block and AF[131] .when the serum magnesium level is > 2.5 mmol/L, the deep tendon reflexes are decreased. Deep tendon reflexes are absent when the serum magnesium level is > 5 mmol/L[132].

Even though some limitation, assessment of serum magnesium concentration is inexpensive and easy to employ and provides important information about magnesium status in patients.

METHODOLOGY

MATERIALS AND METHODS

This research was conducted during the period January 2016 – June 2016 as a comparative cross sectional study in the department of Diabetology and Department of Biochemistry in Govt. Kilpauk Medical Hospital , Chennai

STUDY POPULATION

CASES

50 patients of known type2 DM for ≥ 5 years without any complication will be selected as cases from the OPD of the Department of Diabetology, at Govt. Kilpauk Medical College Hospital ,Chennai.

CONTROLS

Control group comprises 50 normal subjects.

REFERENCE RANGE FOR ZINC AND MAGNESIUM

Serum Zinc – 80 to 120 µg/ dl (12 to 18 µmol/L)

Serum Magnesium – 1.7 to 2.4 mg/dl (0.66 to 1.07 mmol/L)

INCLUSION CRITERIA

Individual between 35 to 60 years with both sexes and they are divided into 2 groups

Group 1- Controls (Normal subjects)

Group 2- patient with type2 DM for ≥ 5 years

EXCLUSION CRITERIA

1. Patient taking any kind of trace element.

2. Hemolysed and jaundiced sample

3. Liver and kidney diseases.

The study was approved by the Institutional Ethical Committee of the Govt. Kilpauk Medical College, Chennai. After giving full explanation of the study a written informed consent was obtained from every participant.

SAMPLE COLLECTION

5 ml of random venous blood sample is drawn from the antecubital vein of the patient in a plain test tube after fulfilling the selection criteria. Serum is separated by centrifugation at 3000 RPM for 10 minutes within 30 minutes of collection .Separated serum is stored at -20◦C for further analysis.

ESTIMATION OF GLUCOSE

Method: Glucose oxidase peroxidase (GOD/POD) (End Point)

Kit Used*:* Erba

Principle

Glucose + O2 + H_2O ⟹ Gluconic acid + H_2O

H2O2 + Phenol+4AAP ⟹ Quinoneimine dye + H_20

The intensity of pink colored Quinoneimine dye is proportionate to Glucose concentration and was measured at 505nm.

Reagent Composition

Reagent 1: Enzyme reagent

Glucose oxidase → ≥ 20000U/L

Peroxidase → ≥ 2000U/L

Phenol → 10 mmol/L

Phosphate buffer → 200 mmol/L

Glucose standard → 100 mg/dl

Procedure

To 1ml of working solution, 10µl of plasma was added and Incubated at 37°c for 15 minutes and absorbance was measured at 505 nm.

Reference range

Fasting plasma glucose: 70 –100 mg/dl

ESTIMATION OF BLOOD UREA

KIT : Accucare

Method : UV - GLDH

Principle

The test is performed as a kinetic assay in which the initial rate of the reaction is linear for a limited period of time. Urea is hydrolysed by urease to NH_3 and CO_2. The NH_3 produced combines with alpha-oxoglutarate and NADH in the occurrence of glutamate dehydrogenase to produce glutamate and NAD.

The initial rate of decrease in absorbance is directly proportional to the urea concentration in the sample. Absorbance is measured at 340nm.

Reagent

Reagent I: buffer reagent

Reagent II: enzyme reagent

Urea standard: 50 mg/dl

Mix 4 parts (4 ml) of buffer reagent with one part (1 ml) of enzyme reagent and mix gently.

Procedure

To 1 ml of the reconstituted reagent 10µl of plasma is added and absorbance measured immediately at 340 nm.

Reference Range

Serum/ plasma Urea → 15- 40 mg/dl

ESTIMATION OF SERUM CREATININE

Kit used : PATHOZYME

METHOD : Jaffe's Method , Initial rate method

Principle

Creatinine in alkaline solution reacts with picrate to form a orange-yellow compound. The color is proportional to the concentration of Creatinine in the sample when measured at 505nm.

Reagent composition

Reagent I : Picric acid reagent.

Picric acid – 25.8 mmol/L

Reagent II: Sodium hydroxide reagent.

Sodium hydroxide – 95 mmol/L

Creatinine standard: 2 mg/dl

Reagents were allowed to attain room temperature. Equal volumes of reagent 1 and reagent 2 were mixed, waited for 15 minutes before use.

Procedure

To 1 ml of the reconstituted reagent 100µl of the plasma was added and absorbance (A1) at 20 seconds after mixing was noted & final absorbance (A2) at 80 seconds were measured.

Calculation

A = A2 - A1

$$\text{Creatinine (mg/dl)} = \frac{\text{Absorbance of Test X concentration of standard (mg/dl)}}{\text{A of standard}}$$

Reference Range:

Males : 0.7 - 1.4 mg/dl

Females : 0.6 - 1.2mg/dl

ESTIMATION OF SERUM MAGNESIUM:

KIT USED: LAB KIT

METHOD : Calmagite – EGTA

PRINCIPLE:

Magnesium forms a purple colored complex when reacts with calmagite in alkaline solution. The intensity of the color formed is proportional to the magnesium concentration in the sample.

REAGENT COMPOSITION

R1	Amino-methyl propanol	1mmol/L
Buffer	EGTA	0.21mmol/L
R2	Calmagite-Chromogen	0.30mmol/L

MAGNESIUM STANDARD - 2 mg/dl

Mix equal volumes of R1 Buffer and R2 chromogen

PROCEDURE

To 1 ml of reconstituted reagent 10 µl of the serum was added. Mix well and incubate for 5 min at room temperature or 3 min at 37◦C. Read the absorbance (A) of the samples and calibrator against the blank.

CALCULATION

Magnesium mg/dl = (A) sample / (A)*2 (calibrator concentration)

REFERENCE RANGE

Serum or plasma – 1.6 to 2.5 mg/dl (0.66 – 0.03 mmol/L)

ESTIMATION OF GLYCATED HEMOGLOBIN (HbA1C)

METHOD: High Performance liquid Chromatography (HPLC)

HbA1C test is done to measure the level of glycated hemoglobin in the blood and reported with eAG- " Average Glucose" . This test shows the mean blood glucose level of the patient for the past 2-3 months.

PRINCIPLE

Done by chromatographic assay using BIORAD D10 HPLC instrument, ion exchange column to separate HbA1C molecules from other hemoglobin molecules. The HbA1C is estimated by the ratio of HbA1C peak area to the total Hb peak areas as given in the equation

eAG(mg/dl) = 28.7* A1C – 46.7

REFERENCE RANGE: Hemoglobin A1C (%)

ADA criteria – HbA1C

Normal - < 5.6%

Impaired - 5.7 – 6.4%

Diabetic - >6.5%

ESTIMATION OF SERUM ZINC

METHOD: AAS (Atomic absorption spectrophotometer)

MACHINE AND MODEL NO: Perkin Elmer 800 AA

PRINCIPLE

Atomic absorption is an emission technique in which an element in the sample is excited and the radiant energy given off is measured as the element returns to its lower energy level. When the light from the hollow cathode lamp enters the flame, some of its is absorbed by the ground state atoms in the flame,

resulting in a net decrease in the intensity of the beam from the lamp. This process is referred to as atomic absorption.

PROCEDURE

Allow the serum samples to come to room temperature and then mix each sample by gently inverting the tube six times. Prepare working standards. Mix 500µl of serum sample to 2 ml of distilled water and mix the solution in the ratio of 1:4.

A Control sample is run in between the patient sample. Aspirate glycerol or water solution in to the luminescent flame and set the baseline to read 0.000±0.001 absorbance (A). Take a baseline reading before and after each sample and reset the baseline as required. At last Zinc values are multiplied by the dilution factor 4.

WAVELENGTH - 213.8 nm

REFERENCE RANGE

80 TO 180 µg/dl

RESULTS

In this study, a total of 100 subjects were enrolled. Out of which 50 were diabetic cases and the rest 50 were controls. They are divided into two groups. Group I includes normal subjects as controls and group II includes diabetic cases.

Serum glucose, S. urea, S.Creatinine, HbA1C, S. magnesium and S. zinc levels were measured in fasting samples of both the groups.

TABLE 1 shows the mean and standard deviation of fasting blood glucose levels between group I controls and group II diabetic patients.

variable	**GroupI(controls) N=50 Mean ±SD**	**Group II (diabetic pts)N= 50 Mean ±SD**	**P Value**	**Statistical significance**
Fasting blood glucose (mg/dl)	90.01±16.67	179.12±73.0	< 0.001	HS

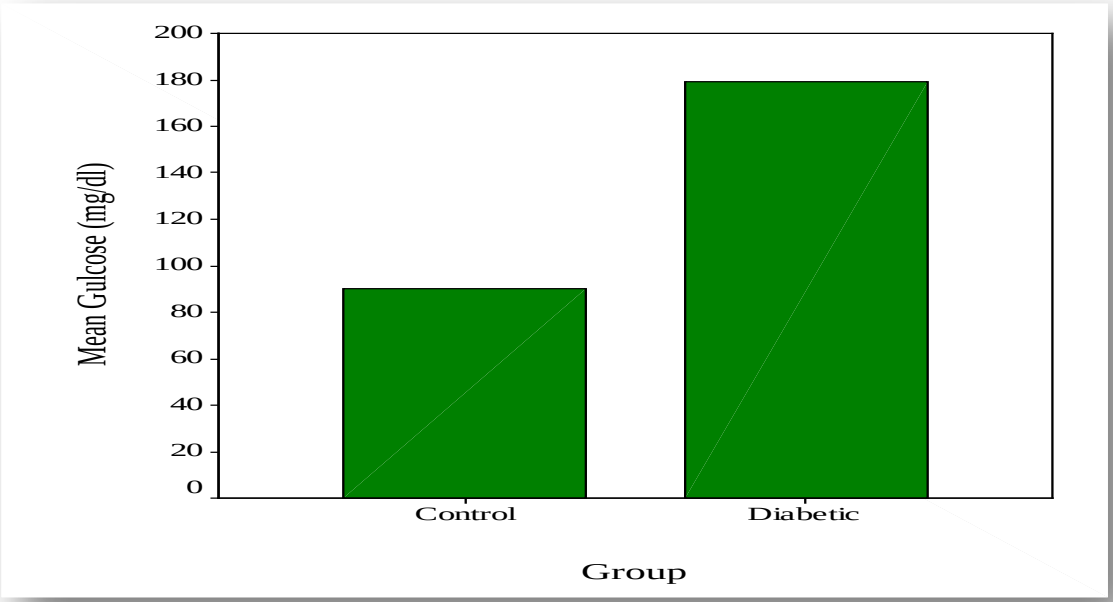

TABLE 2 shows the mean and standard deviation of serum urea of group I controls and group II diabetic patients:

variable	GroupI (controls) N= 50 mean ±SD	Group II (diabetic pts) N= 50 Mean± SD	P value	Statistical Significance
S.urea (mg/dl)	19.36±5.18	21.0±6.69	0.165	NS

NS – not significant

From the above p value (0.165), it is known that there is no statistical significance in urea values between the group I controls and the group II diabetic patients.

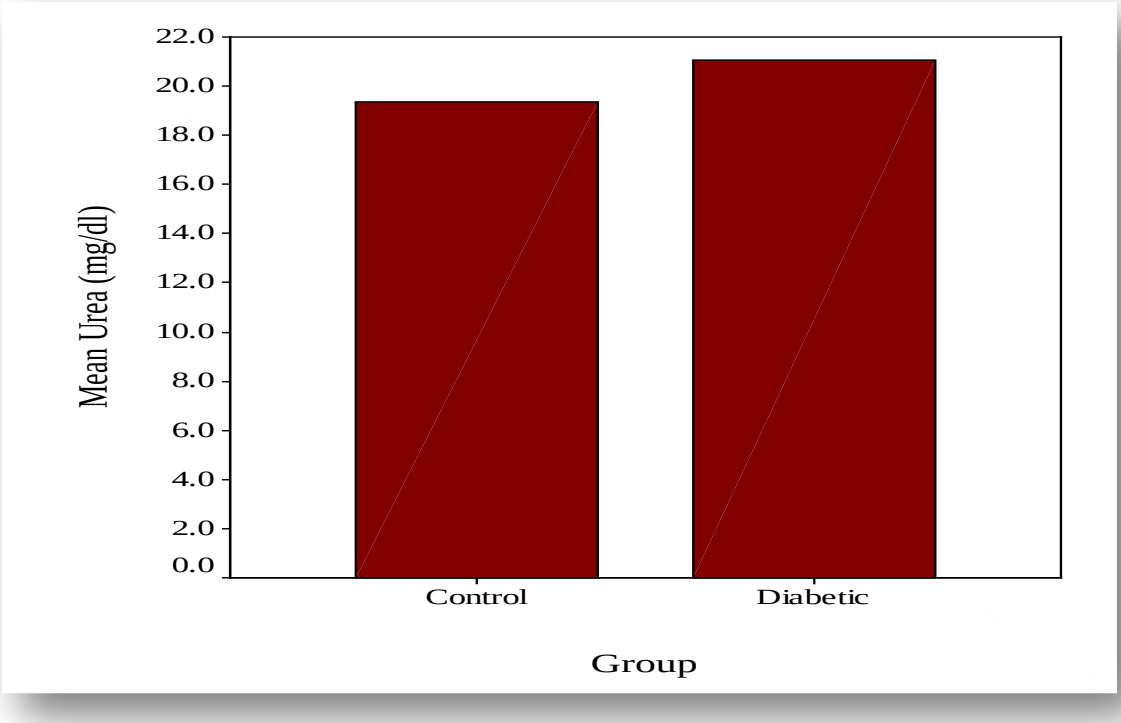

TABLE 3 shows the mean and standard deviation of serum creatinine values between group I controls and group II diabetic patients.

variable	GroupI (controls) N=50 Mean±SD	GroupII Diabetic pts N=50 Mean±SD	P value	Statistical significance
S.creatinine mg/dl	0.85± 0.17	0.80±0.19	0.161	NS

NS = not significant

From the p value (0.161) , it is known that there is no statistical significance in serum creatinine values between the group I controls and group II diabetic patients.

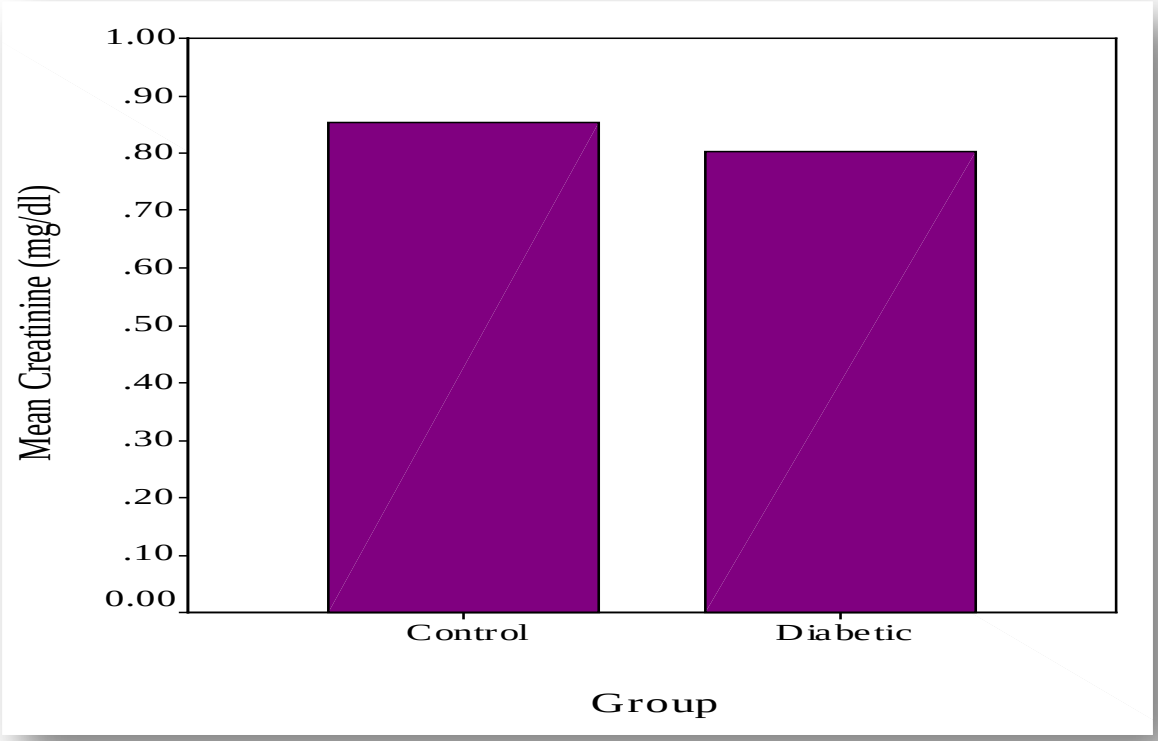

TABLE 4 shows the mean and standard deviation of HbA1C values in group I controls and group II diabetic patients.

variable	Group I (controls) N= 50 Mean±SD	Group II (diabetic pts) N=50 Mean±SD	P value	Statistical significance
HbA1C %	5.44±0.40	8.52±2.14	< 0.001	HS

HS – highly significant

From the table it is known that the HbA1C value were significantly elevated in Group II diabetic patients than the Group I controls.

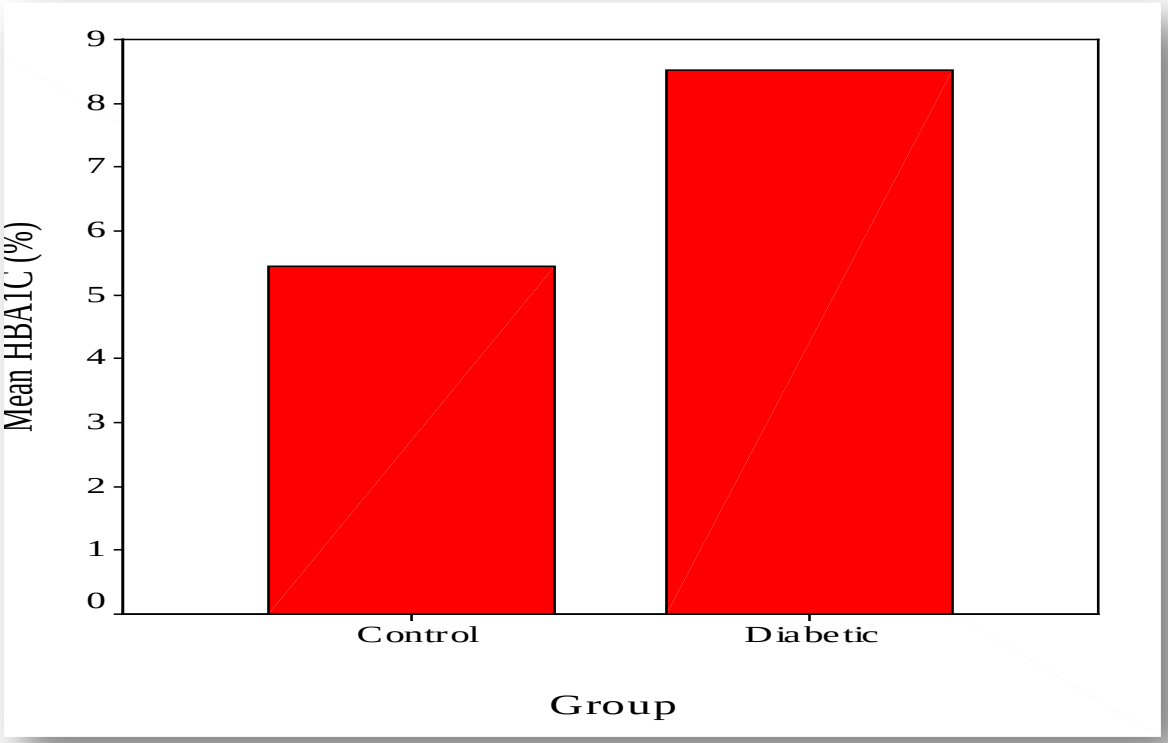

TABLE 5 Shows the mean, standard deviation and p value of serum magnesium in group I controls and group II diabetic patients.

variable	GroupI (controls) N=50 Mean±SD	Group II (diabetic pts) N=50 Mean± SD	P value	Statistical significance
S.Magnesium mg/dl	2.0±0.56	1.56±0.46	< 0.001	HS

HS – highly significant.

From the above table it is known that the serum Magnesium level was significantly lower in group II diabetic patients when compared to group I controls

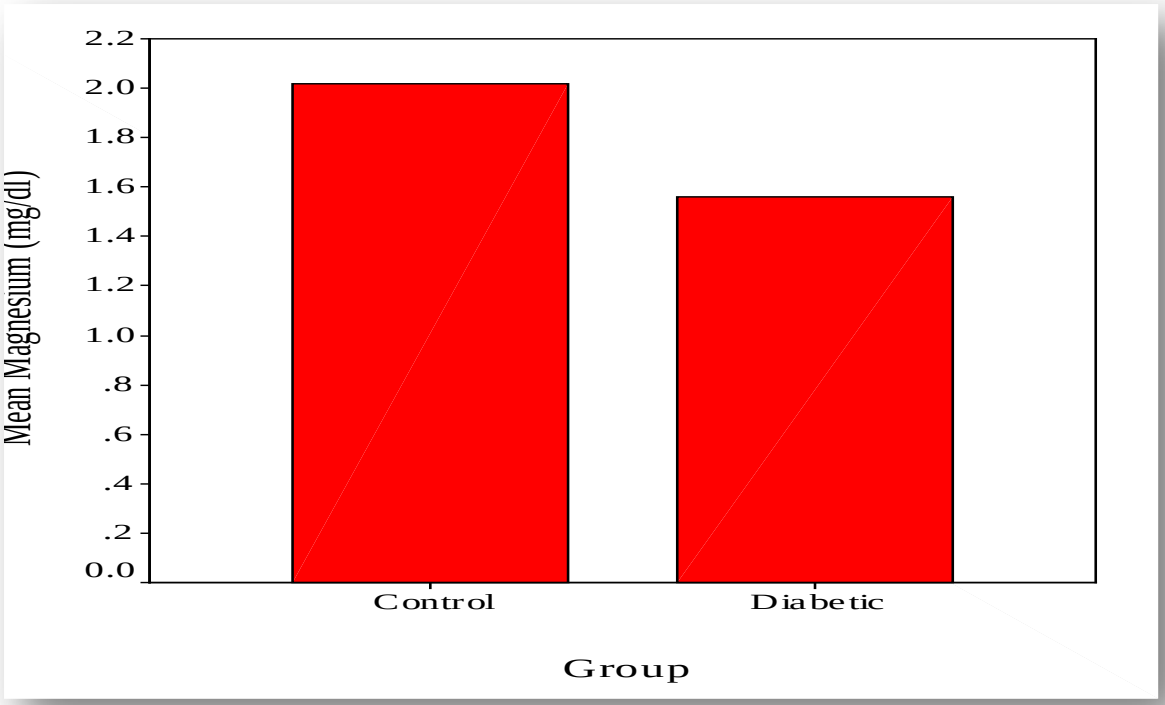

TABLE 6 Shows the mean, standard deviation and P value of serum Zinc levels in group I controls and group II diabetic patients.

variable	Group I (controls) N= 50 Mean± SD	Group II (diabetic pts) N= 50 Mean ±SD	P value	Statistical significance
S. Zinc (µg/dl)	126.84 ± 58.93	122.84±49.61	0.714	NS

NS – not significant

From the above table it is known that the serum zinc levels were decreased in group II diabetic patients when compared to Group I controls but, it was not statistically significant (P value > 0.05)

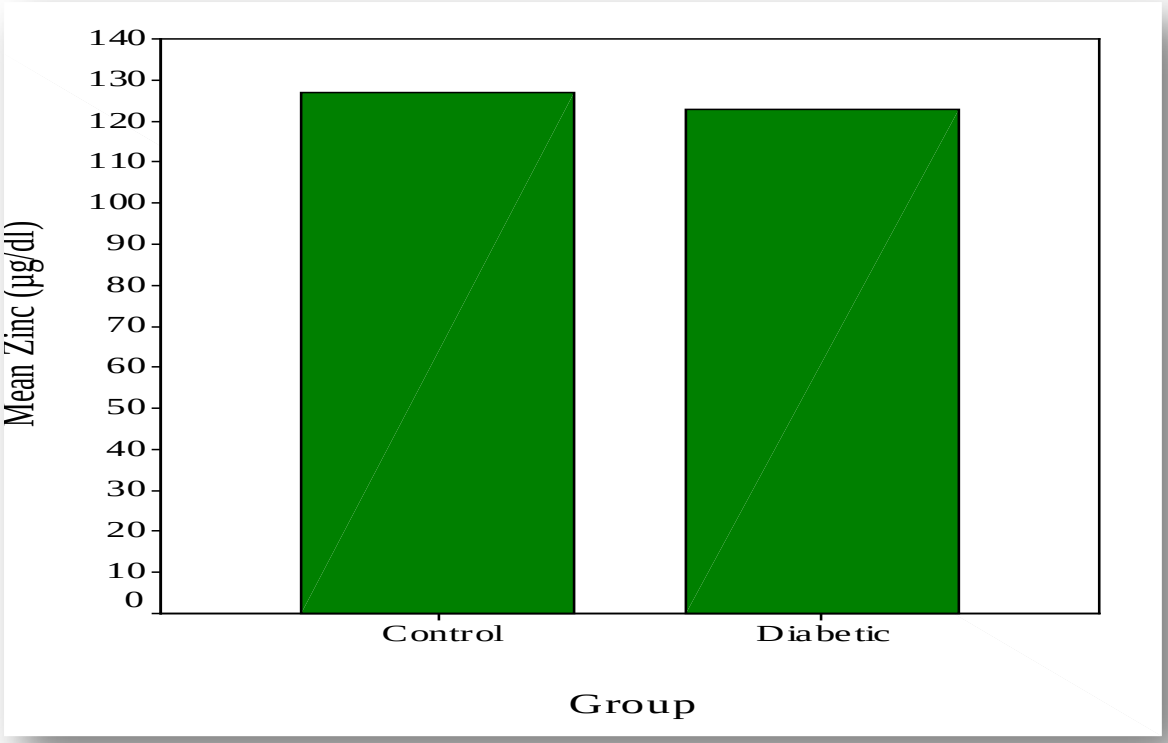

TABLE 7 Shows the correlation of serum magnesium and serum zinc levels with HbA1C in diabetic subjects

Correlation between	**Pearson's correlation Coefficient (r)**	**P value**	**Statistical Significance**
Serum magnesium and HbA1C	-0.56	< 0.01	Highly significant negative correlation
Serum zinc and HbA1C	0.047	>0.05	Not significant

DISCUSSION

This study was done to find an association between trace elements (zinc and Magnesium) and type 2 DM. Zinc and magnesium plays an important role in various metabolic processes of our body.

So in this study the trace elements like serum magnesium and serum zinc levels were measured and its association with glycated hemoglobin was compared between type 2 diabetic patients and healthy non diabetic controls.

Zinc act as a cofactor for insulin. But its mechanism in carbohydrate metabolism is not yet known.

In this study, S.Zinc levels between type2 D M and controls were not significant since the P value is > 0.05 which is consistent with the findings of studies by Zargar et al in Kashmir [133]and Rusu et al in seria[134].

In our study zinc concentrations were similar in diabetic patients and controls. This is consistent with the study Niewoehner et al[6] . There are various other studies that show relationship between DM and serum .Zinc levels. These differences are partly due to heterogeneity in patient selection and study design.

The cause for higher level of serum zinc concentration in diabetic patients is due to the presence of vascular complications according to Rusu et al[134]. They showed that the zinc levels have a moderate but constant increase with obliterative arteriopathy, retinopathy or nephropathy in diabetic

patients[134]. So the abnormality in zinc metabolism is proposed to play a role in pathogenesis of diabetes and its complication.

According to Kinlaw WB et al[135], serum zinc concentrations were decreased in type 2 DM patients and this decrease was due to excessive urinary losses, but this loss was found to be greater in patients when they had proteinuria.In our study we excluded the nephropathy patients.

In our study we excluded the patients who are all having diabetic complications. In another study Schlienger JL et al[136] , found that serum zinc concentrations were reduced in patients with type2 DM and there was no association between zinc and glycated hemoglobin. Control of diabetes did not influence the zinc concentration.

Patients who were previously treated with Insulin showed increase in zinc levels[137]. Our study did not include the diabetic patients who were receiving Insulin.

Fasting sugar, glycated hemoglobin were significantly elevated in type 2 DM patients as compared to healthy controls ($P < 0.001$) where as serum magnesium levels were decreased significantly in DM patients ($P < 0.001$)[138].

DM is one of the cause for hypomagnesemia. In this study, serum magnesium levels in type 2 DM was significantly lower than that of control group (P value < 0.001) and it is negatively correlated with HbA1C.

Various other studies showed that serum magnesium levels are lower in type 1 and type2 DM compared with control normal subjects. Kim DJ et al [139],

they observed the negative correlations between magnesium and HbA1C , fasting blood glucose and HOMA – IR(homeostatic model assessment of Insulin resistance) and this is consistent with our study.

Reduced plasma magnesium level has been shown in NIDDM patients.[140,141]

The cause for hypomagnesemia in type2 DM is not clear. May be due to

1. Indirect hormonal effects
2. Osmotic dieresis [134]
3. Impaired absorption of magnesium
4. Increased urinary losses.

Magnesium has an important role in improving insulin resistance. Decrease in magnesium level in type2 DM patients is mainly due to poor metabolic control or is due to chronic complications according to clinical and epidemiological studies.

The mechanism for magnesium deficiency in diabetic patients have not been clarified, mainly about the effect on insulin resistance and its complications.

Decreased insulin sensitivity due to magnesium deficiency causes alteration of the insulin receptor mediated tyrosine kinase in type2 DM patients[142]. The decreased magnesium level in type 2 DM patients causes increased vascular and adrenal responses to angiotensin II mediated

thromboxane A2 release and increased platelet activity which leads to multiple organ damage from free radical production[143,144].

Our finding of serum magnesium levels and its correlation with HbA1C was similar to the findings of Pujar S et al [145]. The study of Viktorinova et al [146]showed the negative correlation between the serum magnesium and HbA1C in diabetic patients is in agreement with our study.

The altered metabolism of zinc and magnesium in diabetic patients was most probably related to hyperglycemia as indicated by increase in HbA1c level. The altered metabolism of these minerals may be the cause for progression of type 2 DM and its complications.[147]

CONCLUSION

In our study, serum magnesium levels are decreased and there is a negative correlation in the serum levels of magnesium with HbA1c in diabetic patients. Serum zinc levels were similar in diabetic patients and control. No correlation between zinc and glycated hemoglobin.

Hypomagnesemia, is common among type2 diabetic patients. So reduced magnesium level in diabetic patients decreases the insulin sensitivity and increases the risk of complications.

Since zinc acts as an antioxidant, only altered zinc metabolism in diabetic patients were more prone for lipid peroxidation and the complications such as retinopathy, nephropathy and peripheral neuropathy. Here the zinc metabolism is not altered.

Improvement in glycemic control is possible with trace element therapy. Poor glycemic control and its association with type 2 DM patients suggest that the serum zinc and serum magnesium should be a part of the screening procedure in detecting the complications for type2 DM.

So supplementation of zinc and magnesium in type 2DM patients and strict glycemic control can prevent the complications to some extent.

SCOPE FOR FUTURE STUDY

Many studies on the role of magnesium supplementation in type 2 DM patients are recommended in Indian population.

Dietary zinc supplementation has been shown to improve hyperglycemia in animal model only.

So many intervention studies in India should be conducted to improve the status of zinc and magnesium in type2 DM patients and to prevent its complication.

Further studies on the molecular role of zinc and magnesium are needed to confirm its role in causing diabetic complications.

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PATIENT CONSENT FORM

STUDY TITLE: ZINC AND MAGNESIUM LEVEL AND ITS ASSOCIATION WITH GLYCATED HEMOGLOBIN IN TYPE 2 DM

STUDY CENTRE: KILPAUK MEDICAL COLLEGE HOSPITAL,CHENNAI

PATIENT′S NAME :

PATIENT′S AGE :

IDENTIFICATION NUMBER:

I confirm that I have understood the purpose and procedure of the above study.I have the opportunity to ask any questions and all my questions and doubts have been answered to my complete satisfaction

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected.

I understand that the sponsor of the clinical study,working on the sponsor behalf ,the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access .However I understand that my identity would not be revealed in any information released to third parties unless as required under the law. I agree not to restrict the use of any data or results that arise from this study.

I hereby consent to participate in this study.

I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical, radiological tests.

Patient′s name and address: Signature/Thumb impression

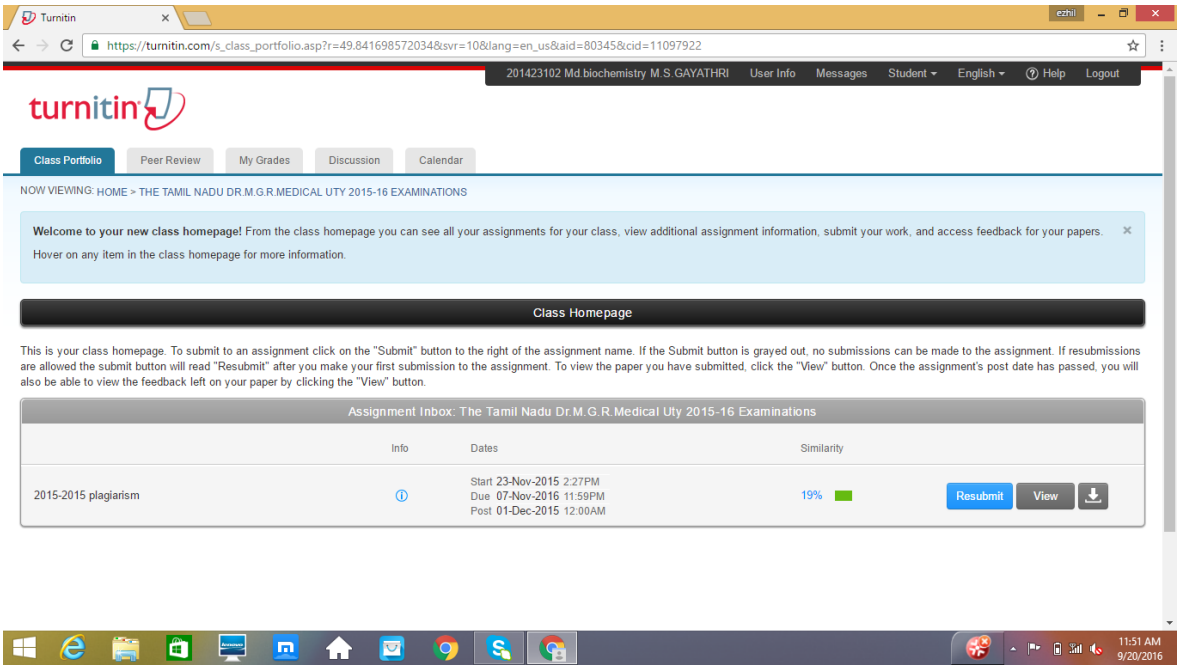
Place:

Date:

Name of the investigator: Signature of the investigator:

Place:

Date: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PROFORMA

Name: OP/IP No:

Age: Sex :

Address: Occupation:

Presenting Complaints: Date:

Past H/O: LIVER AND KIDNEY DISEASES

Treatment H/O:

Previous hospitalization:

Menstrual H/O:

Obstetric H/O: Personal H/O:

O/E:

Built - Height- Weight-

Pedal edema Anemia Clubbing Lymphadenopathy

JAUNDICE

VITALS:

Temperature:

BP: Pulse Rate:

S/E:

CVS: Abdomen :

RS : CNS:

Diagnosis:

Investigations:

Serum – Zinc and Magnesium

Fasting blood glucose

Glycated haemoglobin

Urea

Creatinine

நோயாளி ஒப்புதல் படிவம்

ஆராய்ச்சியின் விவரம்:

ஆராய்ச்சி மையம்:

நோயாளியின் பெயர்: நோயாளியின் வயது:

பதிவு எண்:

நோயாளி கீழ்கண்டவற்றுள் கட்டங்களை (✓) செய்யவும்

1. மேற்குறிப்பிட்டுள்ள ஆராய்ச்சியின் நோக்கத்தையும் பயனையும் முழுவதுமாக புரிந்துகொண்டேன். மேலும் எனது அனைத்து சந்தேகங்களையும் கேட்டு அதற்கான விளக்கங்களையும் தெளிவுபடுத்திக் கொண்டேன். ☐

2. மேலும் இந்த ஆராய்ச்சிக்கு எனது சொந்த விருப்பத்தின் பேரில் பங்கேற்கிறேன் என்றும், மேலும் எந்த நேரத்திலும் எவ்வித முன்னறிவிப்புமின்றி இந்த ஆராய்ச்சியிலிருந்து விலக முழுமையான உரிமை உள்ளதையும், இதற்கு எவ்வித சட்ட பிணைப்பும் இல்லை என்பதையும் அறிவேன். ☐

3. ஆராய்ச்சியாளரோ, ஆராய்ச்சி உதவியாளரோ, ஆராய்ச்சி உபயத்தாரோ, ஆராய்ச்சி பேராசிரியரோ, ஒழுங்குநெறி செயற்குழு உறுப்பினர்களோ எப்போது வேண்டுமானாலும் எனது அனுமதியின்றி எனது உள்நோயாளி பதிவுகளை இந்த ஆராய்ச்சிக்காகவோ அல்லது எதிர்கால பிற ஆராய்ச்சிகளுக்காகவோ பயன்படுத்திக்கொள்ளலாம் என்றும், மேலும் இந்த நிபந்தனை நான் இவ்வாராய்ச்சியிலிருந்து விலகினாலும் தகும் என்றும் ஒப்புக்கொள்கிறேன். ஆயினும் எனது அடையாளம் சம்பந்தப்பட்ட எந்த பதிவுகளும் (சட்டபூர்வமான தேவைகள் தவிர) வெளியிடப்படமாட்டாது என்ற உறுதிமொழியின் பெயரில் இந்த ஆராய்ச்சியிலிருந்து கிடைக்கப்பெறும் முடிவுகளை வெளியிட மறுப்பு தெறிவிக்கமாட்டேன் என்று உறுதியளிக்கின்றேன். ☐

4. இந்த ஆராய்ச்சிக்கு நான் முழுமனதுடன் சம்மதிக்கின்றேன் என்றும் மேலும் ஆராய்ச்சிக் குழுவினர் எனக்கு அளிக்கும் அறிவுரைகளை தவறாது பின்பற்றுவேன் என்றும் இந்த ஆராய்ச்சி காலம் முழுவதும் எனது உடல் நிலையில் ஏதேனும் மாற்றமோ அல்லது எதிர்பாராத பாதகமான விளைவோ எற்படுமாயின் உடனடியாக ஆராய்ச்சி குழுவினரை அணுகுவேன் என்றும் உறுதியளிக்கின்றேன். ☐

5. இந்த ஆராய்ச்சிக்குத் தேவைப்படும் அனைத்து மருத்துவப் பரிசோதனைகளுக்கும் ஒத்துழைப்பு தருவேன் என்று உறுதியளிக்கின்றேன். ☐

6. இந்த ஆராய்ச்சிக்கு யாருடைய வற்புருத்தலுமின்றி எனது சொந்த விருப்பத்தின் பேரிலும் சுயஅறிவுடனும் முழுமனதுடனும் சம்மத்திக்கின்றேன் என்று இதன் மூலம் ஒப்புக்கொள்கிறேன். ☐

நோயாளியின் கையொப்பம் / பெருவிரல் கைரேகை ஆராய்ச்சியாளரின் கையொப்பம்

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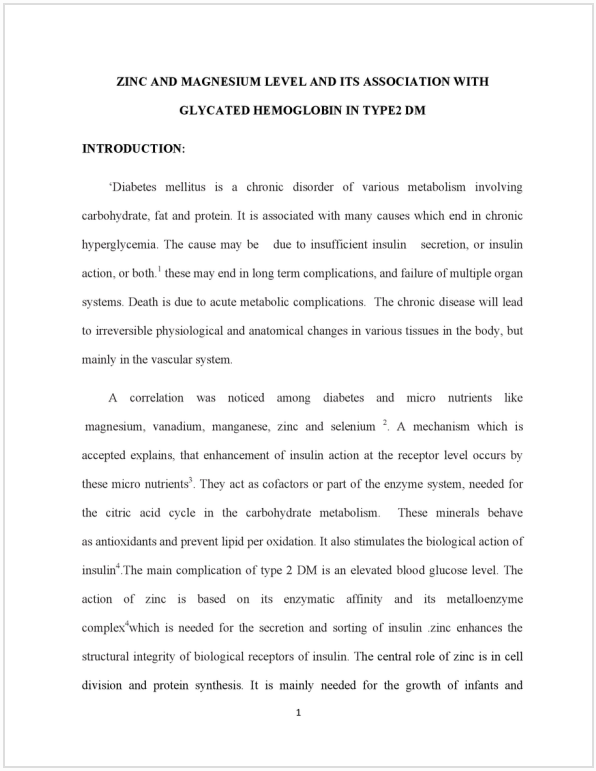

ZINC AND MAGNESIUM LEVEL AND ITS ASSOCIATION WITH GLYCATED HEMOGLOBIN IN TYPE2 DM

INTRODUCTION:

'Diabetes mellitus is a chronic disorder of various metabolism involving carbohydrate, fat and protein. It is associated with many causes which end in chronic hyperglycemia. The cause may be due to insufficient insulin secretion, or insulin action, or both.[1] these may end in long term complications, and failure of multiple organ systems. Death is due to acute metabolic complications. The chronic disease will lead to irreversible physiological and anatomical changes in various tissues in the body, but mainly in the vascular system.

A correlation was noticed among diabetes and micro nutrients like magnesium, vanadium, manganese, zinc and selenium [2]. A mechanism which is accepted explains, that enhancement of insulin action at the receptor level occurs by these micro nutrients[3]. They act as cofactors or part of the enzyme system, needed for the citric acid cycle in the carbohydrate metabolism. These minerals behave as antioxidants and prevent lipid per oxidation. It also stimulates the biological action of insulin[4].The main complication of type 2 DM is an elevated blood glucose level. The action of zinc is based on its enzymatic affinity and its metalloenzyme complex[4]which is needed for the secretion and sorting of insulin .zinc enhances the structural integrity of biological receptors of insulin. The central role of zinc is in cell division and protein synthesis. It is mainly needed for the growth of infants and

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INTRODUCTION:

'Diabetes mellitus is a chronic disorder of various metabolism involving carbohydrate, fat and protein. It is associated with many causes which end in chronic hyperglycemia. The cause may be due to insufficient insulin secretion, or insulin action, or both[1] these may end in long term complications, and failure of multiple organ systems. Death is due to acute metabolic complications. The chronic disease will lead to irreversible physiological and anatomical changes in various tissues in the body, but mainly in the vascular system.

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INSTITUTIONAL ETHICAL COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Protocol ID. No. 19/2016 Dt: 23.01.2016
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "Zinc and magnesium level and its association with glycated hemoglobin in type2 diabetes mellitus" - For Project Work submitted by Dr.M.S.Gayathri, PG Student of MD (BioChemistry), Govt. Kilpauk Medical College, Chennai-10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

DEAN,
Govt.Kilpauk Medical College,
Chennai – 10.

MASTER SHEET

S.NO	AGE	SEX	HBA1C	MEAN_GLUCOSE	GULCOSE	UREA	CREATININ	MAGNESIUM	ZINC
1	48	Female	5.1	100	78.24	14.75	0.8	1.7	106.8
2	45	Male	5.0	97	80.67	15.05	0.7	1.7	372.5
3	38	Female	5.5	111	95.00	18.17	0.8	1.4	113.2
4	30	Female	5.8	120	86.00	19.61	0.8	1.8	88
5	35	Female	5.7	117	70.00	15.30	0.7	2.1	104.8
6	30	Male	4.7	88	72.85	26.88	1.0	2.5	90
7	32	Female	5.4	108	98.00	20.76	0.8	1.3	95.2
8	32	Female	5.9	123	75.00	17.13	0.9	2.4	99.6
9	30	Female	5.0	100	91.32	24.80	0.4	1.5	143.2
10	38	Female	5.4	108	88.00	15.37	0.7	2.1	86.8
11	51	Female	5.3	105	79.68	24.25	1.0	1.7	276
12	30	Male	5.0	97	57.68	17.65	1.2	1.7	95.4
13	37	Male	4.7	88	79.93	16.09	0.9	2.3	118.8
14	33	Female	6.0	124	87.78	14.09	0.7	1.5	194
15	33	Female	5.8	120	79.00	17.31	0.7	2.4	133.2
16	43	Female	4.7	88	85.04	17.24	0.9	2.2	100.4
17	36	Female	6.0	128	82.00	15.24	0.9	2.2	86.4
18	60	Male	4.8	91	97.70	23.02	1.0	1.7	90
19	30	Male	5.8	120	74.57	19.57	0.7	1.4	189.4
20	30	Female	4.7	88	77.54	14.50	0.6	2.5	114.4
21	33	Female	6.0	130	69.00	11.75	0.7	3.2	80.4
22	51	Female	5.4	108	100.00	8.56	0.9	1.9	103.2
23	32	Female	5.2	103	106.20	22.17	0.7	2.1	162
24	58	Female	5.9	133	86.50	30.10	0.8	1.6	137.2
25	37	Female	5.8	120	99.93	14.64	1.0	2.4	118
26	30	Female	4.9	94	79.30	14.46	0.7	1.9	109.2
27	31	Female	5.8	120	117.60	16.24	0.7	2.0	89.2
28	41	Female	5.3	105	94.66	31.77	0.9	4.2	88
29	30	Female	5.3	105	90.40	22.39	0.8	1.7	104.4
30	38	Female	5.4	108	80.27	27.32	0.9	1.9	171.6
31	49	Female	5.7	117	75.60	19.50	1.1	1.8	338
32	48	Male	5.7	117	91.05	21.24	0.9	1.4	124.4
33	45	Female	6.1	128	67.18	17.13	0.7	1.5	106.8
34	52	Female	6.1	128	117.70	19.17	0.8	1.7	119.6
35	45	Male	5.3	105	144.00	24.95	0.7	1.6	88.8

36	31	Male	5.4	108	85.24	21.35	1.0	1.5	102.4
37	47	Female	5.6	114	93.15	21.58	1.3	1.6	108.4
38	33	Male	5.1	100	117.50	15.09	0.7	1.6	121.2
39	44	Female	5.1	100	113.70	28.40	0.8	1.8	102.4
40	46	Female	5.6	114	70.00	21.28	0.8	1.5	95.6
41	54	Female	5.9	123	102.50	33.51	1.0	2.4	104.8
42	30	Female	5.1	100	73.90	16.94	0.9	3.1	132.8
43	30	Female	5.2	103	93.70	14.05	0.8	2.0	84.4
44	30	Female	5.0	97	69.75	18.17	0.9	2.0	109.6
45	40	Male	5.5	111	112.80	17.13	1.0	2.7	130.4
46	57	Female	5.9	123	93.80	18.57	0.9	2.7	91.6
47	43	Male	5.9	123	112.10	18.94	1.1	1.8	97.2
48	43	Male	5.6	114	99.10	23.32	1.3	3.1	105
49	39	Male	5.7	117	108.20	14.35	1.0	2.3	143.6
50	38	Male	5.5	111	99.77	17.24	0.7	1.8	174
51	60	Male	7.7	174	161.30	15.79	0.5	2.2	2.8
52	35	Female	8.6	200	182.80	21.02	0.7	1.6	57.6
53	60	Female	7.7	174	109.20	15.24	0.6	1.6	133.2
54	39	Male	9.8	235	178.30	15.38	0.9	1.7	150
55	60	Female	7.6	171	129.70	23.43	0.9	1.4	133.2
56	54	Female	10.4	252	207.50	19.17	0.6	1.6	114.8
57	55	Female	11.0	269	190.20	15.09	0.7	1.5	321.6
58	58	Male	12.0	298	300.50	28.36	0.9	0.9	140.8
59	46	Female	10.5	255	216.00	19.83	0.9	1.6	175.4
60	55	Female	6.5	140	150.20	21.35	0.5	2.2	188
61	43	Female	6.5	140	107.10	17.35	0.9	2.1	112.4
62	58	Male	10.4	252	188.10	20.65	1.1	0.9	113.6
63	60	Female	7.2	160	145.90	29.44	0.7	1.3	172
64	48	Male	9.0	212	233.40	22.50	0.6	1.3	128
65	60	Female	6.2	131	131.70	13.64	1.0	2.5	100
66	54	Female	10.5	255	132.90	21.28	0.7	1.6	139.2
67	53	Female	7.0	154	125.90	29.55	0.7	1.4	108.6
68	60	Male	11.7	289	300.40	36.55	1.4	1.6	107.2
69	52	Female	9.8	235	343.70	15.98	0.5	0.4	109.6
70	52	Male	15.5	398	438.70	19.13	1.0	0.8	88.4
71	45	Female	8.8	206	127.70	22.76	0.8	1.2	70
72	45	Male	8.0	85	120.00	47.86	1.2	1.4	48
73	49	Female	6.0	126	90.61	19.43	0.9	1.5	66.8
74	60	Male	7.2	160	183.00	13.68	1.0	1.3	164.4
75	42	Female	13.2	323	324.60	16.90	0.9	0.8	128
76	34	Female	6.1	128	126.10	19.06	0.8	1.2	178.4
77	60	Male	11.7	289	325.20	25.14	1.0	1.5	176.8

78	60	Female	6.3	134	163.10	19.57	0.6	2.4	129.6
79	39	Female	9.6	229	251.00	19.65	0.5	1.0	35
80	59	Female	7.8	177	105.70	20.58	0.7	1.5	182.4
81	53	Female	7.0	154	149.40	16.72	0.7	1.5	64
82	53	Female	11.2	275	240.00	25.62	0.8	1.8	130.4
83	45	Female	11.7	289	215.70	19.98	0.6	1.0	118.8
84	59	Female	6.4	137	112.90	18.76	0.9	2.3	115.6
85	57	Male	7.6	171	124.20	24.62	1.0	1.6	130.4
86	60	Female	7.9	180	163.00	24.06	0.8	1.6	143.2
87	50	Female	6.2	128	125.70	17.02	0.7	2.4	156
88	60	Female	8.0	183	204.10	25.21	0.7	1.3	108.8
89	55	Female	10.4	252	238.00	24.99	1.0	1.5	68
90	54	Female	6.8	148	118.20	15.35	0.7	1.5	134.8
91	58	Female	7.3	163	115.80	17.76	0.9	1.6	154.8
92	52	Male	7.0	154	131.60	18.17	0.9	2.1	110
93	55	Female	6.4	137	142.00	15.05	0.6	2.3	116.6
94	38	Female	7.1	157	180.00	17.13	0.8	1.1	162.8
95	46	Female	6.7	146	143.60	35.85	0.8	2.0	110.4
96	46	Female	7.8	177	192.40	17.94	0.6	1.7	63.2
97	56	Female	7.0	154	119.80	28.18	1.2	1.3	142
98	53	Female	7.8	177	123.70	8.23	0.6	1.7	135.6
99	44	Male	8.4	194	162.50	15.24	1.1	1.5	71.2
100	53	Male	7.1	157	163.20	20.61	0.9	2.2	130